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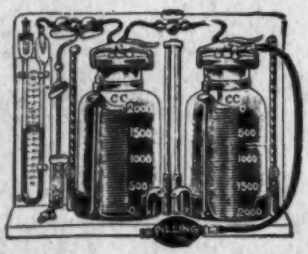
PNEUMOTHORAX



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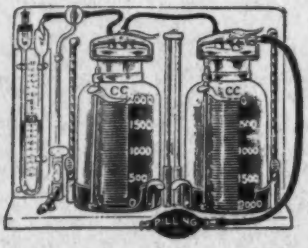

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TUBERCULOUS INFECTION IN PEOPLE DYING OF CAUSES OTHER THAN TUBERCULOSIS[1]

HENRY C. SWEANY,[2] SAMUEL A. LEVINSON[4] AND A. M. S. STADNICHENKO[2, 3]

INTRODUCTION

Few problems in medicine have been more enigmatic than those concerning the source and early course of tuberculous infections. The inability to connect known active cases of tuberculosis with demonstrable sources of contagion caused much confusion and no doubt greatly delayed the discovery of both the infectiousness of the disease and the tubercle bacillus itself. It was recognized long ago that latent periods of months or years frequently exist before the appearance of clinical symptoms. Bayle (1) expressed this idea when he stated that there is a period of the best of health before the actual disease begins. He used the term "occult" to designate active but nonprogressive lesions. Among more recent reports, Rieux (2) used the term "latent" pulmonary tuberculosis to describe the disease that begins as an occult tuberculosis and progresses to incipient disease. Ameuille, Kudelski and Kudelski (3) studied what they called "abortive" tuberculosis. Braeuning (4) described the silent type of disease as *tuberculosis inapparcepta* and Kattentidt (5) as *unbemerkte Tuberkulose*. While latency has been well recognized, it was perhaps not considered as a part of every tuberculous infection, nor was the stormy clinical period interpreted as the end stage of only a minority of tuberculous infections. The majority of cases always remain "latent" and heal in varying periods of time after infection, while the few progressing to activity have a latent period ranging from a few months to two or more decades. Even 30 per cent of "active" cases, according to Braeuning (4), heal spontaneously without being diagnosed. Recently Sayé (6) has ably treated the subject of what might be called "undiagnosed" tuberculosis under the significant title of *Tuberculosis in Subjects Apparently Well.*

The important features of the above mentioned phenomena are their frequent prevalence and the inadequate means of their detection. The first important step in diagnosis was the introduction of the stethoscope by Laennec, but little

[1] The Tuberculosis Institute of Chicago and Cook County granted funds for the purchase of a portable X-ray machine and for the defrayment of a portion of the expenses connected with the bacteriological study and the literature search.

A special research project sponsored by the W.P.A. furnished some of the technical assistance.

[2] City of Chicago Municipal Tuberculosis Sanitarium, Research Laboratories, Dr. Frederick Tice, President of Board of Directors.

[3] Miss Stadnichenko died May 25, 1941. Her work was carried on by her assistants, Messrs. William I. Lansford and John M. Kloeck, and her sister, Miss Vera Stadnichenko, all of whom merit high praise and gratitude. Likewise thanks are due to Miss Alma Everett and assistants for pathological section work; to Mr. Tom Cantalancio for postmortem X-ray films and to Mr. William Martinsen for photographs and illustrations.

[4] Department of Pathology, University of Illinois College of Medicine, Research and Educational Hospital.

progress in physical examination of the lungs was made after that until the sciences of pathology, bacteriology and roentgenology created new foundations on which to establish new criteria.

The attempts to learn about the unknown features have not been lacking nor altogether fruitless, although the problem became involved in controversy with the work of Naegeli (7) and von Behring (8) around the beginning of the century. There was no question about Naegeli's observations, which have been supported by Orth (9), Beitzke (10), Reinhart (11), Burkhardt (12), Puhl (13), Opie (14), Schürmann (15) and others regarding the presence of calcified lesions in practically all lungs of people dying of causes other than tuberculosis. But whether the lesions were generally healed, as Naegeli believed, or were only quiescent, as von Berhing suggested, was to remain a live issue for a generation.

The first efforts toward a solution of the problems involved were directed toward the finding of living bacilli in old lesions of the type described by Naegeli. By inoculating the material into animals, Rabinowitch (16), among many others, found living bacilli in nearly half the calcified lesions and in about two-thirds of soft or chalky lesions. Opie and Aronson (17), however, reported positive findings in only about 30 per cent of all cases. They made the pertinent observation, overlooked by their predecessors, that most soft apical lesions contained tubercle bacilli, whereas focal calcified lesions located elsewhere did not, unless the apical lesion also contained them. In addition they reported 45 per cent positive results in lung tissue away from all focal tubercles. Their evidence apparently supported the contention of Smith (18), and others, that bacilli can live for long intervals in the lungs without causing any tissue reaction. On the other hand, Schrader (19) examined primary tubercles of 41 cases and found only one that produced tuberculosis in guinea pigs (after 10 per cent sulphuric acid treatment of the material). He attributed the low results to the fact that 35 of his cases were over forty years of age. Recently Feldman and Baggenstoss (20) reported finding tubercle bacilli in only 2 of 51 cases (3.9 per cent) in lung tissue and lymph nodes located at a distance from focal tubercles, while only one in 69 tubercles were found positive in an earlier study (21). Their material was selected from patients of all ages, but most of them were around sixty years of age. The authors suggested that their low percentage of positives was probably due to inherent differences in material in different localities. It seems likely, however, that there are additional causes including the universal trends of decreased morbidity and mortality.

This contention is the more credible since laboratory methods for finding tubercle bacilli have continued to improve. The introduction of Ziehl-Neelsen's acid-fast stain, the use of glycerin by Nocard and Roux, Dorset's egg medium, glycerin in egg media by Lubenau, Petroff's direct culturing method with its many improvements, the many forms of sputum concentration and, finally, the examination of gastric contents have permitted an increase in the efficiency of finding tubercle bacilli and should increase rather than decrease the percentage of positive results of tubercle bacilli in tubercles and lung tissue.

Classical pathological methods have been almost equally variable, especially

with regard to the finding of tuberculous lesions in autopsies on the general population.

For example, in spite of the early work of Naegeli and others of that period, Koopman (22) was able to find only 38 per cent tuberculous lesions in 3,041 autopsies from 1919 to 1924. Hart (23), Moenckeberg (24) and Weinert (25), on German soldiers in the last war, were able to find only 30 to 35 per cent with tuberculous lesions. Lubarsch (26), in 3,586 autopsies in various places in Germany, found 36.2 to 69 per cent having tuberculous lesions.

The question of activity of the lesions is even more at variance than the presence of tuberculosis foci, as such. Birsch-Hirschfeld (27), in 826 accidental deaths, reported that 20.7 per cent had tuberculous lesions with 4.2 per cent of them active. Reinhart (11) found 36.1 per cent "unhealed" lesions in adults; Hart (23) found 7.2 per cent active lesions in 573 soldiers; while Robertson (28), in 3,306 autopsies at the Mayo Clinic, found 2,064 cases (62.43 per cent) with some form of tuberculosis, of which 339 (10.25 per cent) had some form of activity. Of the active cases, 89 (2.69 per cent) had tuberculosis as the principal cause of death; 116 cases (3.51 per cent) had associated clinically demonstrable disease and 134 cases (4.05 per cent) were clinically undiagnosed.

In Weinert's 2,394 postmortems on soldiers, 3.4 per cent were considered "active" and 48.9 per cent of a "latent active" type. Guggenheim (29) of Heidelberg, in 7,507 autopsies, from 1918 to 1927, found 72.25 per cent having some type of tuberculosis. Of this number there were 52 per cent cicatricial or calcified, mostly in the apical regions. The remaining 20.25 per cent could perhaps be considered as possessing some type of activity. Caseous encapsulated and calcified foci (Puhl) were present in 5.5 per cent; apical scars with cavity formation in 1.3 per cent; old tuberculosis with signs of more recent progress in 6.6 per cent; isolated caseous or infiltrative foci in 2.1 per cent; extrapulmonary tuberculosis in 2.4 per cent; progressive pulmonary tuberculosis with extrapulmonary lesions in 1.15 per cent, and without extrapulmonary lesions in 1.2 per cent.

The variations of pathological criteria are perhaps due to several causes. Some pathologists may have overlooked many small calcified lesions and especially small infiltrates. There is a great difference in the average age of the cases studied from one clinic to another. Even different terms are assigned to the same phenomenon, especially in different countries, as pointed out by Pinner (30). Since many reports lack accurate and complete description of all the lesions, it is difficult to compare one work with another. There has been insufficient uniformity in the descriptions of the size, location, consistency, pigmentation, thickness of capsule of the lesions; extent of calcification of each tubercle; the character of the perifocal tissue; the condition of related lymph nodes and detailed microscopical structure of the lesions.

In close correlation with the pathology, it is important to consider the possible application made of immune phenomena in the detection of activity. The tuberculin reaction, for example, as performed by Hamburger and Monti (31), Mantoux (32) and D'Arcy Hart (33), parallels closely the pathological statistics

on calcified lesions. Various discrepancies, however, have been observed in X-ray studies. Nelson, Mitchell and Brown (34) reported 16.9 per cent and Crimm and associates (35) reported 21.4 per cent of cases with calcified lesions had negative tuberculin reactions. Lumsden and Dearing (36) and Olson and associates (37) have found ever greater disparity; but they question whether all cases were tuberculosis. Many other less striking results have been reported. At any rate, information at present seems to point to the fact that some lesions heal with a gradual disappearance of the tuberculin reaction. Little is known regarding the duration of tuberculin skin allergy. It is probable that reactivity will last at least as long as that following BCG vaccination, which, according to Aronson and Dannenberg (38), Rosenthal (39) and Ferguson (40), disappears in two to three years. In real infections, however, the bacilli live varying periods of time, even up to decades, and the tuberculin reaction could be expected to survive all infections. At the height of the infection, or shortly thereafter, the tuberculin reaction is perhaps strongest and, as the infection heals, the reaction fades out, only to increase at each recurrence of the infection or in reinfections which are frequent in heavily infected districts. In certain progressive cases the sensitivity may entirely vanish due to desensitization, the development of anergy.

While it appears logical that living bacilli in the body may bear some relation to the degree, if not to the mere presence of the tuberculin reaction, there has been little success or even justification in using the reaction in prognosis or in the determination of activity.

Serological reactions (complement fixation, precipitation, "inhibition reaction" etc.) have also had proponents, but most serological reactions have been entirely abandoned or are held *sub judice,* as indicators of activity.

In the roentgenological field there have been many reports bearing on the presence and activity of tuberculous lesions, many of which have been summarized in a monograph by Sayé (6). There is much variability, however, in the percentage results. Harrington and Myers (41), on school employees, reported radiological activity as follows: first infection 21.7 per cent, doubtful first infection 3.4 per cent, residual serofibrinous pleuritis 2.6 per cent, residual and first infection 1.1 per cent, parenchymatous lesions 3.1 per cent. Of the parenchymatous lesions, 1.4 per cent had discrete unilateral lesions, 0.28 per cent moderately advanced lesions, 1.0 per cent far advanced lesions. One case was an exudative pleuritis.

In a survey in New York City, Edwards (42) found a wide variation in different age, race, occupational and social groups but on the average there was about 4 per cent chronic tuberculosis with 2.5 per cent being "clinically significant." The lowest figures were obtained in college students (0.2 per cent) and high school students (0.4 per cent). The average individuals (teacher applicants, Fire Department applicants, etc.) were found to have 1.0 per cent involvement but among prisoners 4.5 per cent, and among transient men 5.3 per cent were found with "clinically significant" tuberculosis. Long and Hetherington (43) found "probably active" 6.2 per cent and "probably open" 2.7 per cent in a general

survey in Philadelphia. Fellows (44) on 17,947 insurance employees with a mean age of eighteen years, found 1.03 per cent active tuberculous lesions and over that age 3.5 per cent active. Kattentidt (5) reported inactive 21.7 per cent, closed active 0.10 per cent, partly active 0.79 per cent, open 0.37 per cent. Wylie (45) found "radiologic pathology" in 8.2 per cent of Chinese students. Ameuille, Kudelski and Kudelski (3), on prisoners in Paris, found "abortive" type lesions in 3.2 per cent under twenty years and 4.1 per cent, 7.1 per cent, 8.3 per cent and 9.0 per cent, respectively, in subsequent decades. Czezowska at Lemberg (46) reported inactive 17.3 per cent; closed and partly active 5.6 per cent; open 1.6 per cent. Recently, Tice and associates (47) found 4.3 per cent in a survey in a heavily infected district in Chicago's "black belt," while many "white" districts had low rates. It will be noted that the results vary from 0.04 to 2 or 3 per cent for open cases and up to 5 or 10 per cent for "active lesions," depending on the location, age, group, occupation and methods of study used.

In all the branches of medicine pertaining to the question of activity of tuberculosis, there are not only differences in time and place and from author to author, but individuals vary as they advance in knowledge of their subject and gain in experience in the interpretation of their observations. For example, as Naegeli's technique improved, his percentage figures rose for positive findings from 75 to 90, 97 and 98.

In a résumé of the criteria of activity, the earliest method of diagnosis by physical examination has proved inadequate in the recognition of latent active types. Classical findings are only significant if present and are frequently—almost constantly in latent cases—absent. Bacteriological standards have shifted from positive direct smear to sputum concentration, stomach wash and culture, and as a consequence negative findings are limited in value. Immunological data are not dependable at present. Roentgenological criteria have changed from the ability to recognize only massive cloudy or annular shadows to the association of small, soft, round and nebulous lesions with the early evolution of active tuberculosis. The positive percentages as determined by the X-ray methods, even in recent surveys, range all the way from a fraction of one per cent to 10 per cent of active lesions. Likewise the average report for pathologically active lesions from postmortem study is about 4 per cent, but many observers report up to 20 per cent activity. There have been no fundamental criteria established as a basis for the evaluation of positive or negative results.

As significantly pointed out by Opie and Andersen (48) the difference between latent and clinical tuberculosis has "no other basis than limitations of diagnostic methods and the tendency of tuberculosis to proceed to recovery."

One absolute criterion remains, namely, the demonstration of living virulent tubercle bacilli. *The presence of lesion-producing bacilli is the only pathognomonic sign and is prima facie evidence of potential disease.* It follows that the development of disease itself will largely depend upon what happens to the host bearing the living bacilli. When live bacilli are associated in lesions with suspicious pathological activity and when this activity is reflected as different densities or

other special characteristics on X-ray films, the combination may afford a tangible means of foretelling the possibilities in many unknown latent cases and permit us to anticipate and forestall active disease by appropriate measures.

Because of many recent improvements in the technique of finding tubercle bacilli, it was thought feasible and justifiable to try to find the true incidence of all cases having living bacilli in our community; the extent of involvement of such cases, and to discover any clews with respect to the exacerbation of the disease or otherwise to fill in the gaps existing in our present knowledge. Although we made many attempts, it was many years before all necessary procedures became standardized and carried out in proper liaison with each other.

In the last analysis the most desirable information to be obtained is that which will contribute to an earlier diagnosis of the disease and to be able to tell which latent cases will probably become clinically active under varying circumstances of life.

DETAILED OBJECTIVES

The specific objects of the present study were to discover the incidence of active tuberculous lesions, as they exist to-day, in people apparently well; to discover the incidence of the disease in ill people who had no recognized symptoms or diagnosis of tuberculosis; to see whether anything could be determined regarding the mode of development of the disease; to determine whether the development of the disease could be correlated with any states or conditions of the host, such as age, race, sex, occupation or intercurrent diseases; and to see whether any means might be used during life to discover dangerous lesions by correlating histories, clinical, X-ray and laboratory findings. In addition, it was desirable to ascertain, if possible, the relation of the presence of tubercle bacilli to the age and character of the lesions, to other disease processes, and to pathological evidences of active tuberculosis adjacent to or some distance from the focal lesions. Incidentally, the incidence of the completely healed lesions could be obtained by the difference between the total and active lesions. Finally, it was desirable to learn the comparative value, if possible, of different culture methods, acid-fast staining and fluoroscopic microscopy in the identification of living bacilli in tissues.

METHODS

The work involved gross pathological study, postmortem roentgenology, detailed study of histological sections, bacteriological studies and the compilation of the histories.

The method of procedure was so organized that no one group of participating workers could know the results of any other until the final reports were made. The autopsies were performed, the gross and microscopical pathological findings and the presence of extrapulmonary tuberculosis were recorded. In many cases the examination for tubercles preceded the other gross studies.

The various lesions suspected of being tuberculous were charted in their true anatomical position on a stamped outline of the lungs and each lesion was given a number.

The different tuberculous lesions were then removed with sterilized instruments and placed in sterile numbered and stoppered bottles and examined later. Some were studied immediately following removal of the specimens. In either instance the lesions were delivered for study in as sterile condition as possible. In the bacteriological studies, the excess tissue was trimmed off and cultured in 100 of the 300 cases. The practice of culturing perifocal lung tissue was discontinued for reasons to be given later. A few tubercles at the beginning were dipped in alcohol, but this practice was found detrimental to tubercle bacilli.

The tubercle with a small amount of tissue around was cut in two with sterile scissors; one-half was placed in formaldehyde solution for section and the other ground in a sterile mortar to an impalpable magma for culture, smears and animal inoculation. Sometimes the lesions were so large they had to be divided into several parts. The contents of the covered mortar were made into a thin mash with a few cubic centimeters of sterile salt solution and treated in 15 cc. centrifuge tubes for thirty minutes with 4 per cent oxalic acid. Sometimes either 4 per cent sodium hydroxide, 3 per cent hydrochloric acid or 6 per cent citric acid was used. At the end of thirty minutes the material was centrifuged and the sediment planted on two to five culture bottles of two to five different standardized media. The culture media used were Petragnani's; Saenz'; Loewenstein's, modified by Jensen and Holmes; Wooley-Petrick; one of several modifications of the Sweany-Evanoff medium, and occasionally Arenas' medium. Each soft or semisoft lesion was studied separately and cultured, and the majority were inoculated into guinea pigs. Old dense lesions of the same side were occasionally pooled. This economy was justified by the results because few of these "old" pooled lesions were found positive. The cultures were observed weekly after three weeks and were not discarded as negative until six months' time had elapsed. Positive cultures were typed in animals. All animals were killed and examined sixty days after inoculation.

It may be questioned whether the use of all the media was justified, but long experience has taught that too much simplicity fails to give results when only a few weakly viable bacilli are present, as Sweany and Evanoff (49) demonstrated many years ago. All tissues positive on culture were sectioned, stained and examined for tubercle bacilli, but even partial serial sections were of little help.

The microscopical-pathological examination included a detailed description of the various changes in the section, such as caseation, calcification, thickness and type of capsule, with a rough estimation of the age of lesions according to the method outlined in a former study (50). Owing to the facts that half of the lesion was removed for culture and that only one section was examined, there was obviously a limitation as to the accuracy of the estimations. As results show, however, there was an interesting relationship of the assigned age to number of positive cultures. The smears from young lesions were examined completely. All old lesions were studied for at least fifteen minutes and some for one to two hours to find the maximum number of bacilli. Smears were also saved for fluorescent microscopical examination to test the efficiency of the fluorescent

method for such material and also to add another control. The results of this comparison will be reported later.

Each lesion was described accurately with respect to its physical character and located with regard to its relationship to the other lesions, lymphatics, bronchi, pleura and blood vessels. In this way the course of the infection in the various lung and lymph node lesions could be reconstructed. Most of the time there was a simple primary complex, consisting of one or a few lesions in the parenchyma, with the corresponding involvement of the chain of lymph nodes towards the hilum to and through the thoracic duct to the lungs and finally through the capillaries of the lungs to the general circulation involving especially the spleen and liver by haematogenous dissemination. Other cases had a recent focus in one or both apices; others showed isolated recent foci located indiscriminately in any part of the lung, while still others revealed definite reinfection complexes. A few were too complicated to classify at all by any known means.

The laboratory work was done without any knowledge concerning the clinical status of the patient. All the clinical, gross pathological and antemortem X-ray data were assembled separately.

Much of the information pertaining to the histories of the patient and roentgenological interpretations, as well as elaboration of other features of the work must necessarily await the final report.

EXPERIMENTAL RESULTS

This preliminary report involves only the first 300 completed cases of the total 1,000 already begun, but only partly studied at this date. The lung specimens were obtained from the Coroner's Laboratory and a few of the General Hospitals of Chicago.

In table 1 the cases were arranged according to decades and in three groups with respect to the presence of, or the type of calcified lesions. The greatest number of cases (24.1 per cent) were in the sixth decade with a progressive drop to less than 5 per cent in the first and a similar drop in the late decades (table 1).

Only 7 per cent of the first and 55 per cent of the second decade had densities thought to be calcified primary lesions. Allowing 10 per cent for extrapulmonary infections, saturation of the population with first infection, when judged by the presence of X-ray densities in the lungs, could be placed in the early part of the fourth decade (chart 1). It is probable that in certain districts the percentage infection per age period would be lower and complete saturation would not occur until later. The results must be accepted largely as those of the lower economic strata of a large midwestern American city.

In table 2 there is a breakdown of the whole series into various aspects of the disease. First, there are the cases having definite tuberculosis as judged clinically or by acceptable gross anatomical standards, in which no pathognomonic signs were presented before death. There were 9 cases (4.3 per cent) which would be universally adjudged as "obvious" or "frank" tuberculosis on the basis of quantity and quality of lesions present. They were divided into two groups: one group consisted of 5 cases where either extrapulmonary disease was present,

TABLE 1

The studied material arranged in three groups according to the findings in the lungs and separated into decades, each of which was divided as to sex

	DECADES																				TOTAL	
	0–10		11–20		21–30		31–40		41–50		51–60		61–70		71–80		81–90		91+			
	Sex																					
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
No calcification	7	6	3	2	4	0	3	5	1	3	3	3	5	1	0	0	0	0	0	0	26	20
																						46
Small lesions (unstudied)	0	0	1	1	1	5	4	3	4	2	8	1	5	3	4	0	0	0	0	0	27	15
																						42
Larger lesions studied	1	0	3	1	8	7	23	6	26	12	40	17	34	11	14	5	2	2	0	0	151	61
																						212
Totals	8	6	7	4	13	12	30	14	31	17	51	21	44	15	18	5	2	2	0	0	204	96
	14		11		25		44		48		72		59		23		4		0			300
Per cent cases per decade	4.67		3.67		8.33		14.67		16.00		24.00		19.67		7.67		1.33		0			100
Per cent calcified lesions per decade	7		55		80.4		82		92		92		89.7		100		100					

or where some clinical impression of tuberculosis had been gained some time earlier, but no bacilli were ever found; and the second group consisted of 4 cases where no X-ray films had been taken and all tests were negative, except gross and microscopical pathological findings. In a preliminary report (51) the combined

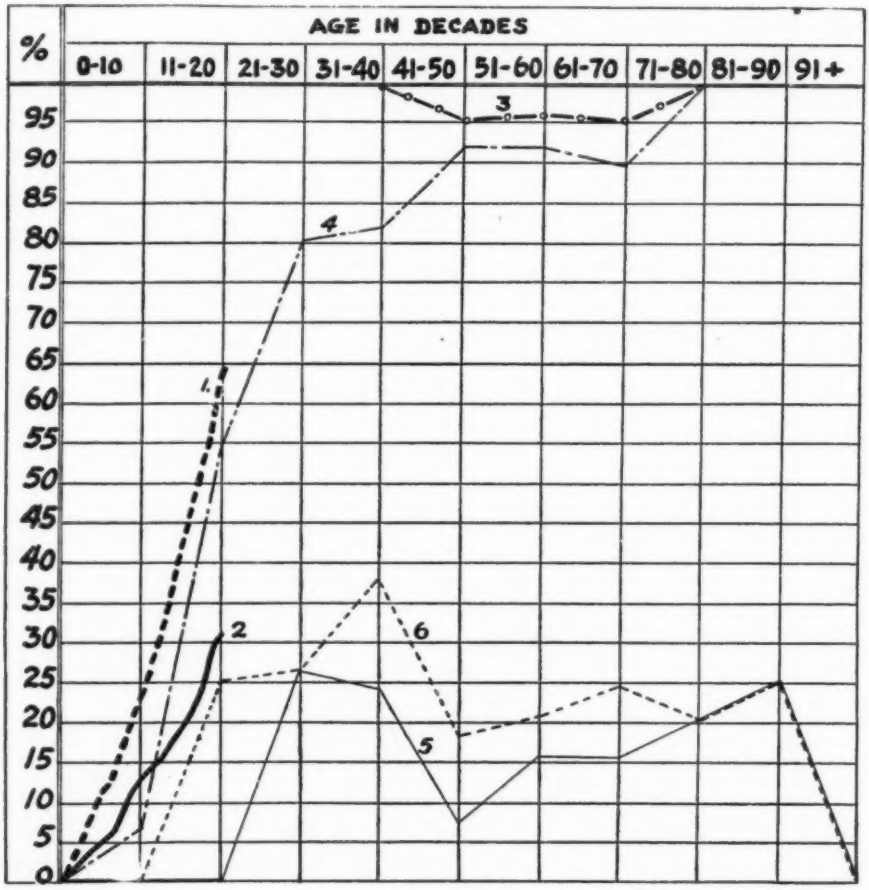


CHART 1

1. — · — — · — Tuberculin-positive colored children in Chicago (47).
2. ———— Tuberculin-positive white children in Chicago (47).
3. —○— Tuberculin findings in adults, by Kereszturi, *et al.* (52).
4. — — — Calcified lesions in series of 212 cases.
5. ——— Positive cultures. The cause of the low beginning is due to the fact that there was only one calcified lesion in the first decade which was negative; and 4 in the second decade with only one having positive pathological signs.
6. - - - - Total positives including acid-fast stains and pathological findings.

figure was given as 3 (1.4 per cent) but a more thorough study of the histories and lesions compelled the rearrangement cited above.

There was a total of 245 cases having either lesions or obscure signs of activity designated "occult" and lesions entirely healed, of which 42 were too small to be

divided successfully with scissors. There were 12 cases in the latter group which had from one to 20 lesions, each 1 mm. or less in diameter. They were thought to be haematogenous disseminations from an unknown extrapulmonary primary focus. Thirty cases had a few or many lesions from 1 to 2 mm. in diameter. The majority of the remaining 203 lesions were from 2 to 5 mm. in

TABLE 2

Division of cases on the bases of roentgenological and pathological evidence

	NUMBER OF CASES STUDIED	TOTAL NUMBER OF CASES	PER CENT OF THE 212 CASES STUDIED		PER CENT OF ALL 300 CASES		
I. Obvious tuberculosis							
A. Clinical pulmonary activity found or suspected by physical or X-ray examination, or both, or diagnosis of extrapulmonary tuberculosis, but with no report of acid-fast bacilli in the sputum. Cases 61, 62, 65, 71, 143	5	5	2.4		1.7		
B. No clinical activity described (or no X-ray film made) but gross and microscopical tuberculosis found. Cases 169, 179, 225, 251	4	4	1.9	4.3	1.3	3.0	
II. Occult and healed pulmonary tuberculosis—245 cases						81.7	
A. Lesion 1 mm. or less		12			4.0		
B. Lesion 1 to 2 mm.		30			10.0		
C. Lesion 2 mm. or more which were studied	203	203		95.8	67.7		
Total number with densities thought to be tuberculous lesions—254 cases							84.7
III. Number tuberculous pulmonary lesions found		46			15.3	15.3	15.3
Total	212	300		100.00	100.00	100.00	100.00

diameter. There was a gradual decrease in number of larger lesions with a few 3 to 4 cm. in size. Forty-six specimens had no pulmonary lesions visible on the roentgenograms. By adding this number to the 42 cases with lesions too small to study successfully, it made a total of 88 unstudied specimens (29.3 per cent). The remaining 212 cases (70.7 per cent) were acceptable for study and consisted of 9 "obvious" or "frank" and 203 "occult" or healed lesions.

The results of the search for the presence of tubercle bacilli in the 212 cases studied are shown in table 3. The findings of acid-fast bacilli and positive cultures are recorded. There were 7.6 per cent positive for acid-fast bacilli and 17.0 per cent positive cultures. Twenty cases (9.4 per cent) were positive on culture alone. Two (1 per cent) were considered positive by the acid-fast examination alone because so many typical acid-fast bacilli were present with compatible pathological changes. Three cases, negative by bacteriological methods, had so many signs of pathological activity that they were considered positive. Two other cases of an established extrapulmonary tuberculosis were also included. In 14 cases both smear and culture were found positive, giving a total of 44 positive cases or 20.8 per cent of the studied cases. There were 9 cases wherein acid-fast bacilli were found, but neither corresponding living bacilli nor pathological activity were present. Of the 9 cases, 6 had only one bacillus per slide, 2 cases had two bacilli, and one had three bacilli per slide. It seems highly

TABLE 3

Active cases found by bacteriological and pathological methods

	POSITIVE ON SMEAR	POSITIVE ON SMEAR ONLY	POSITIVE ON CULTURE	POSITIVE ON GUINEA PIG	POSITIVE PATHOLOGY ONLY	TOTAL POSITIVE
76 in first 100	5		10		3*	13
Per cent	6.6		13.1			17.1
66 in second 100	3	1	11		1	13
Per cent	4.6		16.7			19.6
70 in third 100	8	1	15	1	1	18
Per cent	11.4		21.4			4.3
212 of a total 300	16	2	36	1	5	44
Per cent	7.6		17.0			20.8

* Includes 2 cases of extrapulmonary tuberculosis with negative lung findings.

probable that these few bacilli are dead or saprophytes. In 2 definitely positive cases only acid-fast granules were found.

The total number of colonies on the culture bottles was with few exceptions from ten to fifteen times as great as the number of acid-fast bacilli found in smears of the same specimens. Since about ten times as much material was used for cultures as for smears, the results were fairly comparable.

It must be remarked that we do not claim to have found every living bacillus or even every positive case. In fact, we are reasonably certain that 2 cases (perhaps more) had living bacilli which were not found. That is why several of the best media were used wherever possible. The variations of growth, however, shown in table 4, illustrate the results and necessity for intensive culturing. Twenty of the cases had fewer than 25 colonies of bacilli—some only one to 3—which would have been missed had not all the tubercles been studied and had not many good media and many culture bottles of each medium been used. But

as the material studied is of the most difficult from which living bacilli may be grown, even a 10 per cent error in specimens having only one to a few feebly growing bacilli would still be acceptable.

In a test for virulence, a few of the cultures failed to kill guinea pigs in 1.0 mg. dosage of moist bacilli. None of the cultures affected rabbits. The bacilli which failed to affect guinea pigs were either living or dead human type tubercle bacilli or mere saprophytes. Owing to the fact that many cultures were temporarily misplaced for a long interval of time, it is feared that some may have died out before they were injected. In the final analysis this feature will be more completely reported because the cultures are now injected as soon as a good growth is obtained.

There were 4 cultures of avirulent yellow, semi-acid-fast bacilli which proved to be saprophytes; they were not counted in the positive findings.

In addition to the inconstancy of bacteriological results, there was not always agreement between the pathological lesions and the cultures and smears. It must be recalled, however, that the parts saved for section were not cultured and vice versa, and that tubercles may have colonies of bacilli only in one side. The sections made involved only one small portion of the half saved for section, while the culture represented practically all the half that was cultured. For the same reason it may also be possible to have positive cultures without evidence of recent tubercle formation. While bacilli may possibly live for some time entirely encapsulated, the presence of bacilli without tissue reaction was not often found except in silicotic and cancer tissue (table 3). Evidently the time of survival in pathologically inactive lesions is with few exceptions of a relatively short duration (plate I).

There is one important question which these findings have helped to answer, namely, the relation of the presence of living tubercle bacilli in the body to the tuberculin reaction. Judging by an extensive literature on tuberculin reactions, people over fifty years of age in large cities are found to range from 70 to 100 per cent positive. The work of Kereszturi (52), Bréton (53) and LaBlaye (54) is representative. As the work reported here shows a minimum of 16.99 per cent and a maximum of 20.75 per cent with living bacilli, it follows that only about one in four or five people having a positive tuberculin reaction also have living bacilli in their bodies (chart 1).

In table 4 are detailed the results in a group of 9 representative cases, with respect to type of case, the bacteriological details and the pathological findings. There is no uniformity of results regarding the different media used. First one medium, then another gave best results. It would seem that there are other factors than the medium, such as very fine differences in gas tension and moisture. For all types of human tubercle bacilli, however, the Saenz medium has thus far been unsurpassed in efficiency of results and simplicity of preparation.

CASE REPORTS

The case bearing X-ray *No. 54* was a forty-six year old white male who died of endocarditis. He had a 4-plus Kahn reaction. Of a cluster of many lesions in both upper lobes,

TABLE 4

Bacteriological and pathological findings in the tubercles of 9 representative positive cases

X-RAY NUMBER	HISTORY	TUBERCLE (T.) AND TISSUE (TIS.)	CULTURES*	SMEARS FOR ACID-FASTS	VIRULENCE TEST: 1.0 MG. IN GUINEA PIG; 0.1 MG. IN RABBIT	PATHOLOGY OF TUBERCLE	ESTIMATED AGE OF LESION IN YEARS
54	Forty-six year old white male; endocarditis; Kahn 4-plus	T1	+ 6 col. (3) 5 col. #3 1 col. W-P. 0 col. S.	−	+guinea pig −rabbit from T1 #3	Thick capsule	10
		T2	+++ (3) ++++ #3 4 col. S. 1 col. W-P.	−		Thin capsule	6
117	Fifty-seven year old white male; blacksmith; pericarditis	T1	−	+(1 bac.)		Giant cells in capsule	
143	Twenty-three year old Negro female; meningitis	T1	−	−		Partly disintegrated capsule	
		Tis. T1	+ 36 col. (2) 30 col. S. 6 col. L.	+(3 bac.)			
		T2A	Contaminated			Early bone formation	12
		T2B	+ 38 col. (2) 20 col. S. 18 col. L.	+		Epithelioid tubercle	2
		Tis. 2	+ 2 col. (2) 1 col. S. 1 col. L.	−	+guinea pig −rabbit from tissue 2		
		T3	− (2)	−		Eroded capsule	12
		T4A	− (2)	−		Monocytic giant cells	1
		T4B	Contaminated	−		Eroded capsule	15
		T4C	− (2)	−		Heavy capsule Monocytic capsule	7 3

						Monocytic capsule	3
169	Eighty-four year old white male; "pensioner"	T1	++ (3) +++ P. 12 col. #3B 5 col. S.	+(3 bac.)	+guinea pig −rabbit (pooled cultures)	Silicotic	
		T1A	−	−		Silicotic	
		Tis. T1, T1A and T1A	++ (5) +++ S. 10 col. #1 0 col. P.L.3B				
		T2A	++++ (5) S.P.L. 1, 3B				
		T2B	+ 30 col. 20 col. S. 10 col. P. 0 col. L.	+(8 bac.)		Silicotic	
		T2C	++++ (5) S.P.L. 1, 3B	+(25 bac.)		Silicotic	
		T3	+++ (5) S.P.L. 1, 3B	+(1 bac.)		Silicotic	
			++ 84 col. (4)† 37 col. L. 30 col. S. 15 col. #1 2 col. P.	+(15 bac.)		Silicotic	
170	Thirty-seven year old white male; pneumonia	T1A	+ 9 col. (4) 7 col. L. 1 col. S. 1 col. #1 0 col. P.	−		Thick capsule	10
		T2	+ 1 col. (4) 1 col. #1	−			
			Others negative		Lost	Thick capsule	8
		Tis. T2	+ 3 col. (4) 2 col. S. 1 col. #1	−			
		T1B, T3	Others negative				
		T4, T5, T6	All negative	−			
		Tis. T3					

TABLE 4

Bacteriological and pathological findings in the tubercles of 9 representative positive cases

X-RAY NUMBER	HISTORY	TUBERCLE (T.) AND TISSUE (TIS.)	CULTURES*	SMEARS FOR ACID-FASTS	VIRULENCE TEST: 1.0 MG. IN GUINEA PIG; 0.1 MG. IN RABBIT	PATHOLOGY OF TUBERCLE	ESTIMATED AGE OF LESION IN YEARS
54	Forty-six year old white male; endocarditis; Kahn 4-plus	T1	+ 6 col. (3) 5 col. #3 1 col. W-P. 0 col. S.	−	+guinea pig −rabbit from T1 #3	Thick capsule	10
		T2	+++ (3) ++++ #3 4 col. S. 1 col. W-P.	−		Thin capsule	6
117	Fifty-seven year old white male; blacksmith; pericarditis	T1	−	+(1 bac.)		Giant cells in capsule	
143	Twenty-three year old Negro female; meningitis	T1	−	−		Partly disintegrated capsule	
		Tis. T1	+ 36 col. (2) 30 col. S. 6 col. L.	+(3 bac.)			
		T2A	Contaminated			Early bone formation	12
		T2B	+ 38 col. (2) 20 col. S. 18 col. L.	+		Epithelioid tubercle	2
		Tis. 2	+ 2 col. (2) 1 col. S. 1 col. L.	−	+guinea pig −rabbit from tissue 2		
		T3	− (2)	−		Eroded capsule	12
		T4A	− (2)	−		Monocytic giant cells	1
		T4B	Contaminated	−		Eroded capsule	15
		T4C	− (2)	−		Heavy capsule Monocytic capsule	7 3

						Monocytic capsule	3
169	Eighty-four year old white male; "pensioner"	T1	++ (3) +++ P. 12 col. #3B 5 col. S.	+(3 bac.)	+guinea pig −rabbit (pooled cultures)	Silicotic	
		T1A	−	−		Silicotic	
		Tis. T1, T1A and T1A	++ (5) +++ S. 10 col. #1 0 col. P.L.3B				
		T2A	++++ (5) S.P.L. 1, 3B				
		T2B	+ 30 col. 20 col. S. 10 col. P. 0 col. L.	+(8 bac.)		Silicotic	
		T2C	++++ (5) S.P.L. 1, 3B	+(25 bac.)		Silicotic	
		T3	+++ (5) S.P.L. 1, 3B ++ 84 col. (4)† 37 col. L. 30 col. S. 15 col. #1 2 col. P.	+(1 bac.) +(15 bac.)		Silicotic Silicotic	
170	Thirty-seven year old white male; pneumonia	T1A	+ 9 col. (4) 7 col. L. 1 col. S. 1 col. #1 0 col. P.	−		Thick capsule	10
		T2	+ 1 col. (4) 1 col. #1 Others negative	−	Lost	Thick capsule	8
		Tis. T2	+ 3 col. (4) 2 col. S. 1 col. #1	−			
		T1B, T3 T4, T5, T6 Tis. T3	Others negative All negative	−			

TABLE 4—*Continued*

X-RAY NUMBER	HISTORY	TUBERCLE (T.) AND TISSUE (TIS.)	CULTURES*	SMEARS FOR ACID-FASTS	VIRULENCE TEST: 1.0 MG. IN GUINEA PIG; 0.1 MG. IN RABBIT	PATHOLOGY OF TUBERCLE	ESTIMATED AGE OF LESION IN YEARS
225	Twenty-eight year old Mexican male; homicide; in "good health"	T1A	+++ 320 col. (4) 183 col. W-P. 93 col. S. 25 col. P. 19 col. 3B	8 acid-fast granules	+guinea pig Pooled T1ABCDE	Thin capsule	4–8
		T1B	Negative	−			
		T1C	Negative	−			
		T1D	+ 6 col. (4) 2 col. S. 2 col. P. 1 col. 3B 1 col. W-P.	Lost		Monocytic capsule	3
		T1E	++++ 451 (4)	Few acid-fast granules	+guinea pig	Monocytic capsule	2
		T2	− (4)	−	from T2	Bony lesion	10–12
		Tis. T3	− 2 col. (2) 1 col. S. 1 col. P.	−			
		T3 (not examined)					
255	Seventy-eight year old white male; malignant nephrosclerosis	T1	− (3)	−	−	Old heavy capsule	10–12
		Tis. T1	+ few col. (3) Few col. #1 0 S. 0 L.	−	−	Eroded bony lesion	15
		T2	+++ 260 (3) 110 col. L. 85 col. #1 75 col. S.	+(5 bac.)	+guinea pig from T2	Eroded bony lesion	15
		T3A, T3B } Pooled	++++ 600 (3)	+(8 bac.)		Eroded bony lesion	15
		T4	Negative	−			

271	Twenty-nine year old Negro female; syphilis, died of Herxheimer's reaction	T1 and 2	− (3) P.L. *1	−		Heavy capsule	12
		T3	− (3) P.L. *1	−		Thick capsule	10–12
		T4	+ 15 col. (3) 10 col. L. 3 col. *1 2 col. P.	+(2 bac.)		Epithelioid capsule	3–5
		T5	++ 82 col. (3) 30 col. P. 27 col. L. 25 col. *1	Lost		Thin capsule	5
		T6	++ 97 col. (3) 50 col. P. 28 col. L. 19 col. *1	+(3 bac.)		Thin capsule	5
		T7	+ 3 col. (3) 3 col. L. 0 col. *1 0 col. P.	−		Thin capsule	5
		T8A	++ 37 col. (3) 21 col. L. 10 col. *1 6 col. P.	Lost		Thin capsule	5
		T8B	+ 12 col. (2) 11 col. L. 1 col. P. 0 col. *1	+(1 bac.)	+guinea pig from T8A and 8B	Thin capsule	5

TABLE 4—*Concluded*

X-RAY NUMBER	HISTORY	TUBERCLE (T.) AND TISSUE (TIS.)	CULTURES*	SMEARS FOR ACID-FASTS	VIRULENCE TEST: 1.0 MG. IN GUINEA PIG; 0.1 MG. IN RABBIT	PATHOLOGY OF TUBERCLE	ESTIMATED AGE OF LESION IN YEARS
293	Thirty-seven year old white male; eight years a roofer	T1A	++++ 700 col. (2)	+(1 bac.)		Silicotic	
		T1B	++ 85 col. (2) 50 col. S. 35 col. W-P.	+(2 bac.)		Silicotic	
		T1C	++ 70 col. (2) 40 col. W-P. 30 col. S.	(1 bac.)	Lost	Silicotic	
		T2A	+ 13 col. (2) 7 col. S. 6 col. W-P.	Lost		Silicotic	
		T2B	+ 2 col. (2) 1 col. S. 1 col. W-P.	−		Silicotic	
		T4A	+ 10 col. (2) 8 col. W-P. 2 col. S.	+(1 bac.)			
		T4B	+ 3 col. (2) 2 col. S. 1 col. W-P.	−		Silicotic	
		T3	Negative	−		Silicotic	

* The media included Loewenstein (L), Petragnani (P), Woolley-Petric (W-P), Saenz (S), #1, #3 and #3B.

"Col." signifies colony; the number in parenthesis represents the number of media used.

The first plus signs are averaged from the individual results which are recorded immediately below, with the number designated by a figure and letter.

The significance of the plus signs are, + = 1 to 15 colonies; ++ = 16 to 100 colonies; +++ = 101 to 300 colonies; ++++ = 301+ colonies.

† Treated with sodium hydroxide instead of oxalic acid.

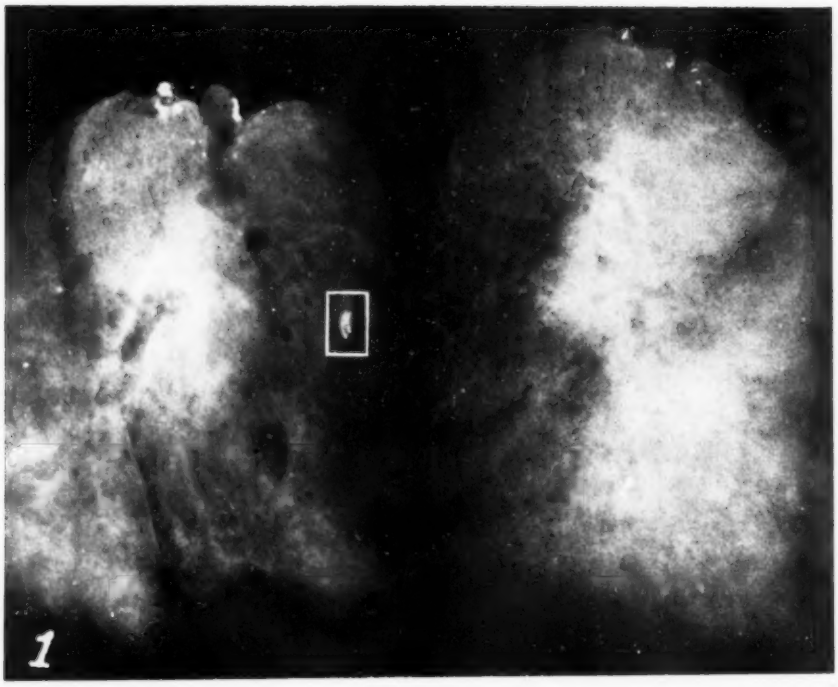
only those at the apices contained tubercle bacilli. From the lesions in the right upper lobe, from which tubercle 1 was taken, only six colonies grew in all, while in the left apex, from which tubercle 2 was taken, a variety of growths were obtained, namely, one colony grew in the Wooley-Petrick medium, four colonies on Saenz medium, and a 4-plus growth appeared on the No. 3 medium—a modified Sweany-Evanoff medium (55). Since an injected guinea pig died of tuberculosis but not a rabbit, the bacillus is of human type. The lesions in the left lung were older than those in the right (see plate I).

Case No. 117 was a fifty-seven year old blacksmith who died of pericarditis. There was a primary complex consisting of small tubercles of at least a ten-year age type and one large round infiltration (Albert focus). The large focus revealed a peripheral progression of activity around the tubercle with giant cells in the outer layer. This type of lesion has been termed an "overflowing type." One acid-fast bacillus was found in the smear (see plate II). All cultures were negative.

Case No. 143 was a twenty-three year old female Negro who had a slowly progressive pulmonary disease following a primary infection. The extension was predominantly in the lymph nodes. In the largest node there were processes of three distinct ages, as the extension progressed towards the hilum. There was a great variation in the character of the lesions. For example, tubercle No. 1—the parenchymal primary—seemed to be disintegrating because of an encroachment of the surrounding disease on the outside of the capsule. No acid-fast organisms by smear or culture were found. From the tissue around the tubercle, however, 35 colonies grew on two media and 3 acid-fast bacilli were found on smear. Tubercle No. 2, consisted of two parts, one (*a*) an old lesion with early bone formation, and (*b*) a recently encapsulated peripheral portion with giant cells and thin capsule (see plate III); *a* became contaminated on culture and *b* grew 38 colonies on two media. The residue from *a* and *b* was pooled and found to be positive on smear. From the tissue around the focus grew 2 colonies but it was negative on smear. The bacilli were virulent for guinea pigs. Tubercle 3 was similar to 2. On one side giant cells were present, but there were old parts to the lesions as in tubercle 2. Cultures and smears of the tubercle and tissue were all negative. Tubercle No. 4 in the right hilum nodes consisted of three distinct parts—a lower part consisting of disintegrating islands of dense calcification, a middle part of solid calcification and an upper part near the carina consisting of soft putty-like caseous material. There was a capsule over the soft part of about a three-year age type. Bacteriological activity was not demonstrated, but there was revealed an unmistakable recent change, pathologically characterized by a slow progression in the direction of the lymph flow (plate III).

This case was chosen principally to illustrate the progressive spread in the lymph nodes (as well as in the parenchyma). In tubercles 2, 3 and especially 4, there was an endogenous progression that was unmistakable because it took place beneath the capsule of the lymph nodes. The younger lesions were immediately adjacent to the next older ones and were progressing with the lymph flow, that is, the most recent lesions were located closer to the hilum (table 4).

No. 169 was an eighty-four year old "pensioner" whose detailed history was not available. The lesions were all silicotic and the findings are self-explanatory, except that tubercle 3 was divided and one part was treated with 4 per cent oxalic acid and the other with 4 per cent sodium hydroxide. The oxalic acid treated specimen grew better than the alkali treated one, but many more acid-fast bacilli were found on smears made from the latter. There is no explanation for this difference.



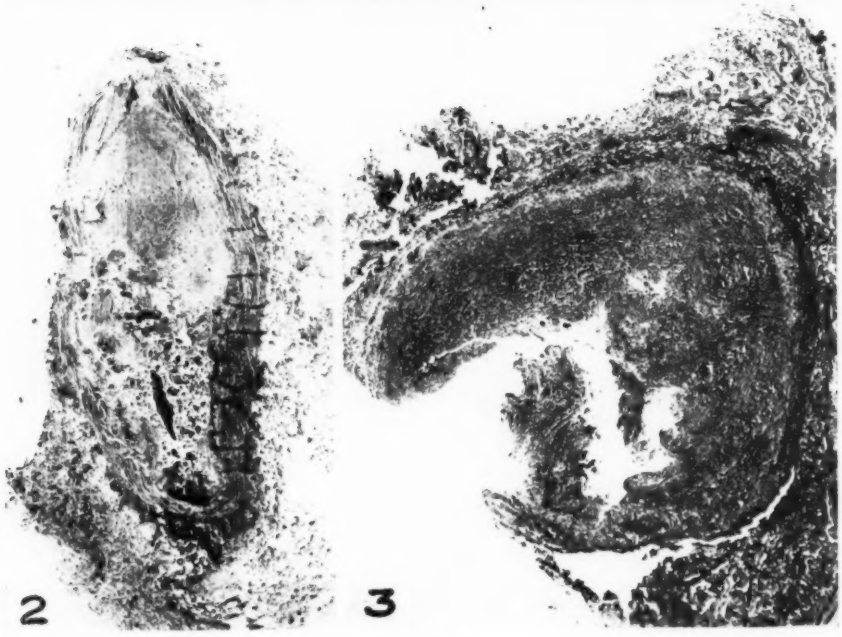


PLATE I

FIG. 1. Postmortem roentgenogram of lungs of No. 55. Note the old bilateral dense lesions in both bases. Both lungs had small lymph node lesions at the hilum as well as both apices. There are two separate age types, as shown in figures 2 and 3.

In figure 2, shown in rectangle of figure 1, there is a densely calcified core of the tubercle with a heavy hyalinized capsule, while in figure 3, from the apex of the opposite lung, the capsule is thin and the core is not so densely calcified. Living bacilli were recovered from both apices, but not from any of the older lesions below. × 8 and × 23

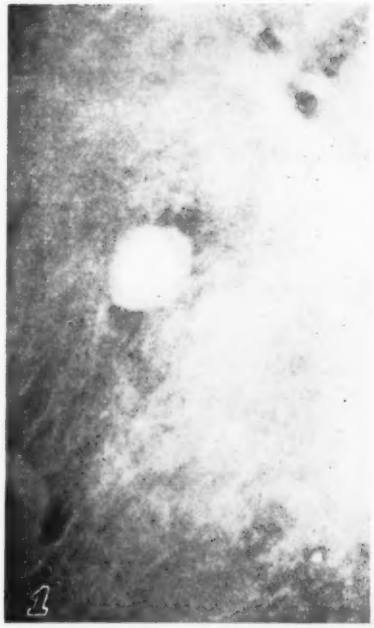


PLATE II

FIG. 1. A "spot" picture from a postmortem roentgenogram of No. 117, showing a round infiltrate with a soft border. Also many smaller lesions.

FIG. 2. A low power photomicrograph of the large lesion. × 5

FIG. 3. A high power of the border of the lesion showing giant cells in the outer capsule. × 290

In *case No. 170*, a thirty-seven year old laborer who died of pneumonia, a few colonies were grown from each of several tubercles. The smears were all negative. In specimens having less than 15 colonies, acid-fast bacilli are rarely found on direct smear. The tubercles

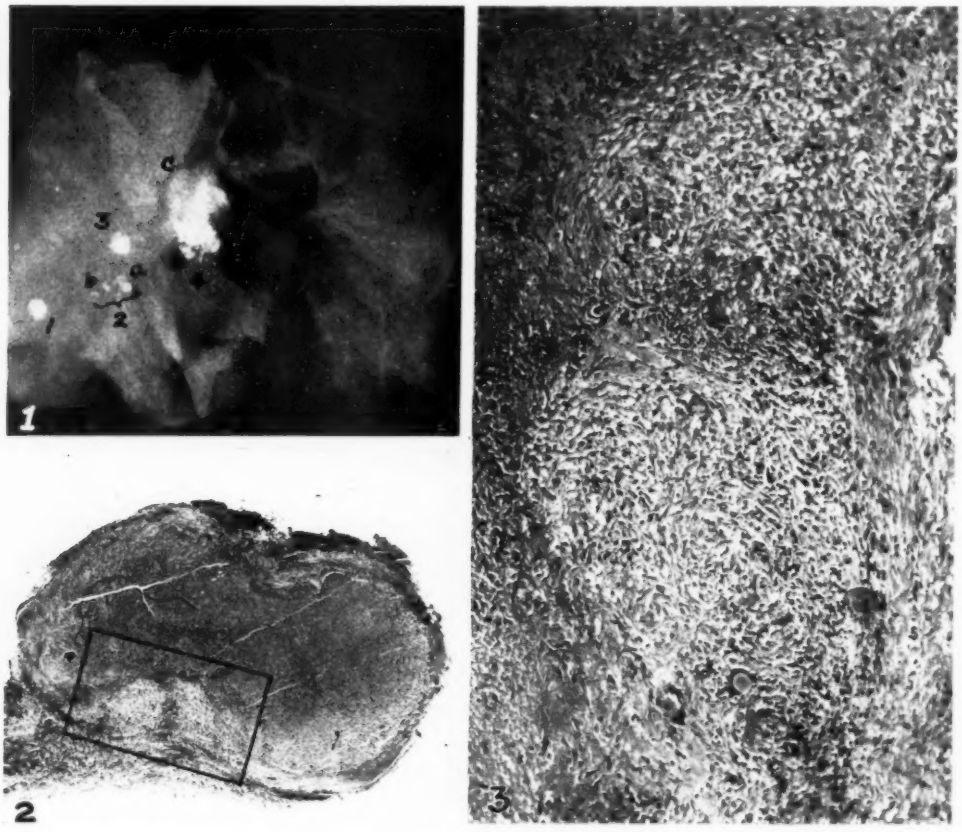


PLATE III

FIG. 1. A postmortem roentgenogram of No. 143, showing a massive primary lesion undergoing a slow healing and simultaneous progression. No bacilli were found in lesions 1, 2, 3 and 4 (2a was contaminated) but 2b (see figures 2 and 3) and the tissue around tubercle 1 had good growths of tubercle bacilli. The tissue around tubercle 3 contained many giant cells. Lesion 4 had at least three distinct ages from old islands of dense calcification to soft lesions, similar to 2b.

FIG. 2. Photomicrograph of lesion 2b or the small lesions around the old densely calcified lesion at 2. × 18

FIG. 3. Enlarged segment of figure 2. × 108

were fairly old. The cultures might have become avirulent on standing for several months and failed to kill either guinea pigs or rabbits.

No. 225 was a twenty-eight year old Mexican apparently in good health up to the time he met a homicide death. The roentgenological shadows and both gross and microscopical pathology revealed a ten to twelve year old lesion apparently of a primary type located in

the apex and lymph nodes near the hilum. There was a gradation of lesions downward towards the base of the lobe with decrease in the density of the calcification; density of the infiltrative shadows, thickness of the capsule, a changing from a calcareous to caseous and then to a fibrous and monocytic type of lesion, as well as an increase in the size of the infiltrates. It is perhaps a slow and coarse dissemination of the type described by Loeschcke (55). An interesting feature was that only acid-fast granules were found and not typical acid-fast bacilli (see plates IV and V).

No. 255 was a seventy-eight year old male who had malignant nephrosclerosis and ten to fifteen year type tuberculous lesions. There was nothing to indicate any recent exacerbation in the tuberculous lesions, yet the cultures were strongly positive. There may have been a very recent infection on an old healed process.

No. 271 was a twenty-nine year old Negress with a recent tuberculous infection who had received treatment for syphilis. She died of a Herxheimer's reaction. The principal disease process was in the left lung and the old and new lesions were in close proximity to each other. The recent infection may have been either a reinfection complex or a progressive infection in the lymph nodes. Cases such as this one must remain in doubt until a more certain basis for classification is available.

No. 293 was a thirty-seven year old white man who had worked eight years as a "roofer"—laying paper, tar and rock dust on the roofs of buildings. There was a first-stage silicotuberculosis consisting of hundreds of small silicotic tubercles throughout both lungs, with "egg shell calcifications" (56) in the lymph nodes around the hilum. The findings suggested that tubercle bacilli had perhaps been disseminated haematogenously in large numbers and that, contrary to the usual healing of the foci in the absence of silicosis, most of them developed into silicotic tubercles. Whether the fibrotic foci would have developed without the bacilli or whether the silica was responsible for their development is problematical. This conception is in keeping with the experimental findings of Gardner (57) and Kettle (58), who showed that silica acts as a stimulant to the growth of tubercle bacilli in the tissues. The bacilli did not produce tuberculosis, however, but appeared to remain imprisoned in the nodules (see plates VI and VII).

The gaps in table 4 were due to the fact that some of the smears were not recovered after Miss Stadnichenko's death. The cultures were likewise misplaced, and, when ultimately found, several failed to grow satisfactorily. It was, therefore, a question whether the cultures were dead or avirulent.

Table 5 gives a summary of the results obtained by culture of the individual lesions studied. The total number of cultures were tabulated and the percentage positive findings calculated. There were 751 separate cultures made with an average of two to five media of two to five bottles each—a total of over 7,500 separate inoculations.

An approximation of the ages of the lesions was made and the positive results were recorded for each age group. Although all the age estimations were made on the basis of those worked out formerly (50) for primary tubercles with application to all types of lesions, there is still a significant relationship between the estimated age and percentage of positive culture growths. Because of the fact that the total number of the first two years is rather low, not much emphasis is to

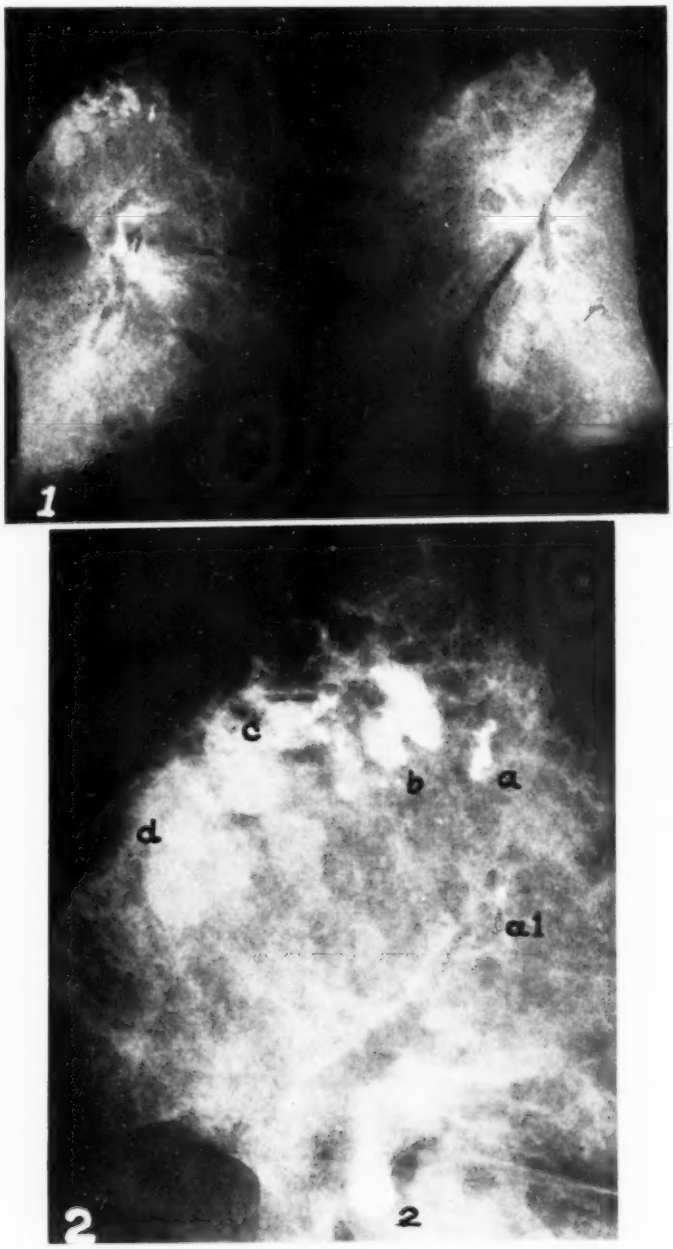
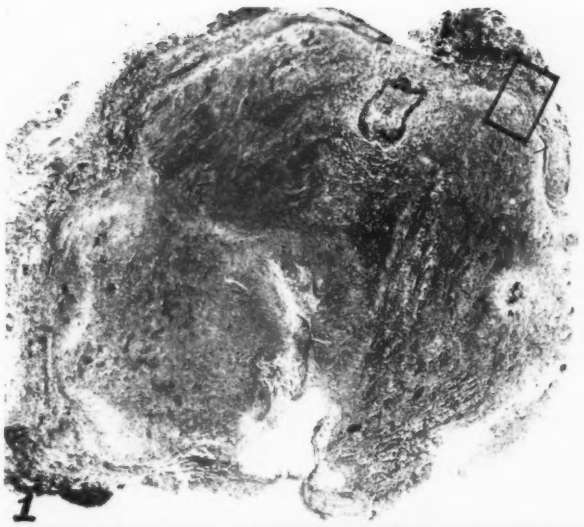
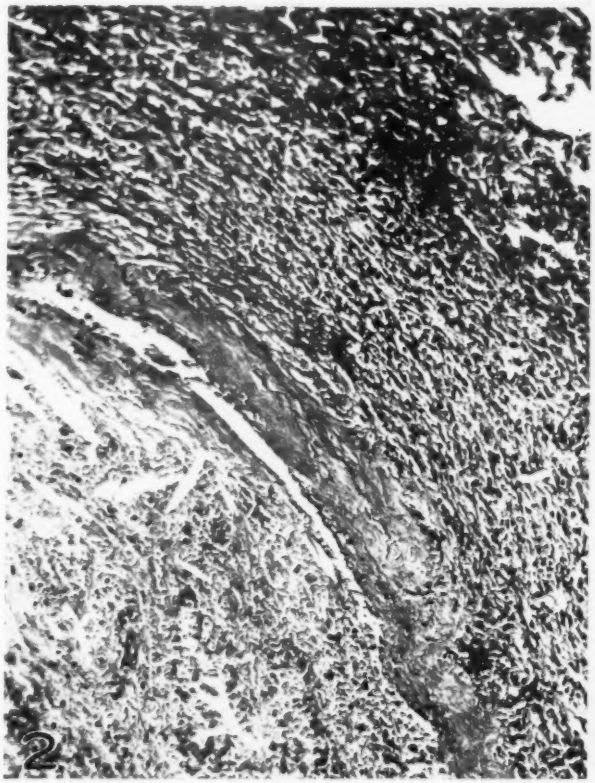


PLATE IV

FIG. 1. Roentgenogram of lungs of No. 225, showing the apico-caudal progression of an old primary lesion in the right upper lobe.

FIG. 2. Enlarged upper lobe of figure 1a. Note the densest lesions a, a1 and 2; a similar cluster of dense lesions at b but with the difference that there are large peripheral "soft" areas. At c there is a large infiltrate with a small calcified centre. At d there are only vague infiltrates.





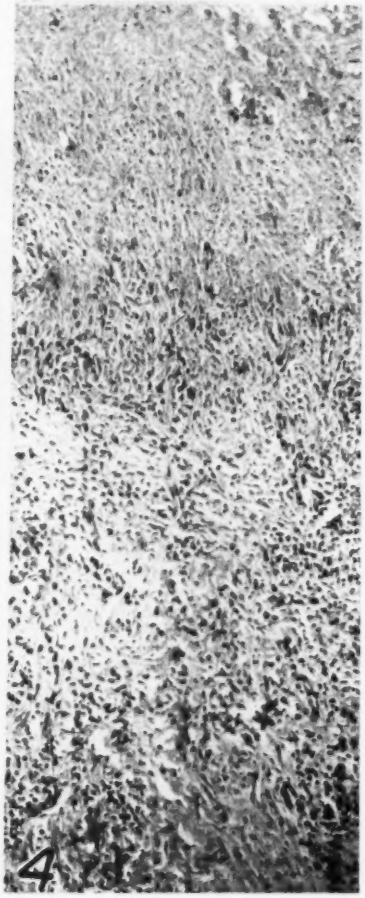


PLATE V

FIG. 1. A large tuberculous mass shown on plate IV, figure 2c. × 8

FIG. 2. An enlarged segment showing a capsule with a partly caseous centre, a "monocytic" type capsule, and an outer capsule. × 135

FIG. 3. A low power of a segment of the infiltrate in plate IV, figure 2d.

FIG. 4. An enlargement of the border of figure 3 showing a loose fibrosis in the capsular layer.

be placed on these early periods. When it is observed, however, that all of 127 specimens with ages estimated at sixteen years or over were negative, and of 159 cases from eleven to fifteen years only 6.3 per cent were positive, the estimations take on a significance worthy of confidence. An arrangement of the series on the basis of *positive cases*, with only the lowest age of tubercle in each case instead of total tubercles, was more striking. The latter arrangement gave percentage figures of 80, 50, 40, 28.6, 36.4, 10.9, 6.10 and 0 respectively, for the age groups of lesions shown in table 5. This feature will be elaborated upon in the final report.

The study of the tissue around tubercles gave interesting results and is worthy of reporting in some detail because of the prominence already given this work.

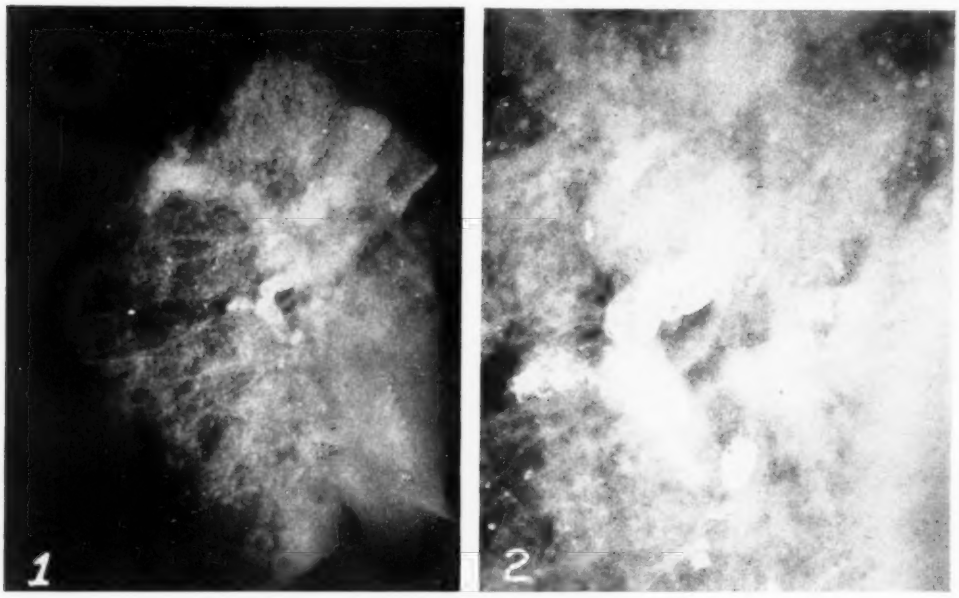


PLATE VI

FIG. 1. The right lung showing a haze of fine densities and large lymph node lesions at the hilum.

FIG. 2. An enlargement of the hilum showing innumerable 1 mm. calcifications in the lung parenchyma and egg-shell calcifications at the hilum.

Opie and Aronson (17) reported positive findings in uninvolved tissues in almost half of their specimens—in fact higher than in the tubercles themselves—while Feldman and Baggenstoss (20) found very low results—3.92 per cent in tissues and 1.45 per cent in tubercles. We studied 100 specimens by separating the tubercle from the surrounding tissue and obtained 11 positive and 89 negative tissues. Since the technique varied slightly as we progressed, some avoidable discrepancies may have been introduced. At first the lesions were trimmed down to one or two millimeters of the capsule, dipped in alcohol for fifteen to thirty seconds to kill any air-borne contamination. It was soon found, however, that not only is air-borne contamination a negligible factor, but the alcohol treatment

appeared to kill the few remaining tubercle bacilli which, as we have since learned, are prone to be harbored in the outer part of the capsule.

Each one of these positive results will be described briefly.

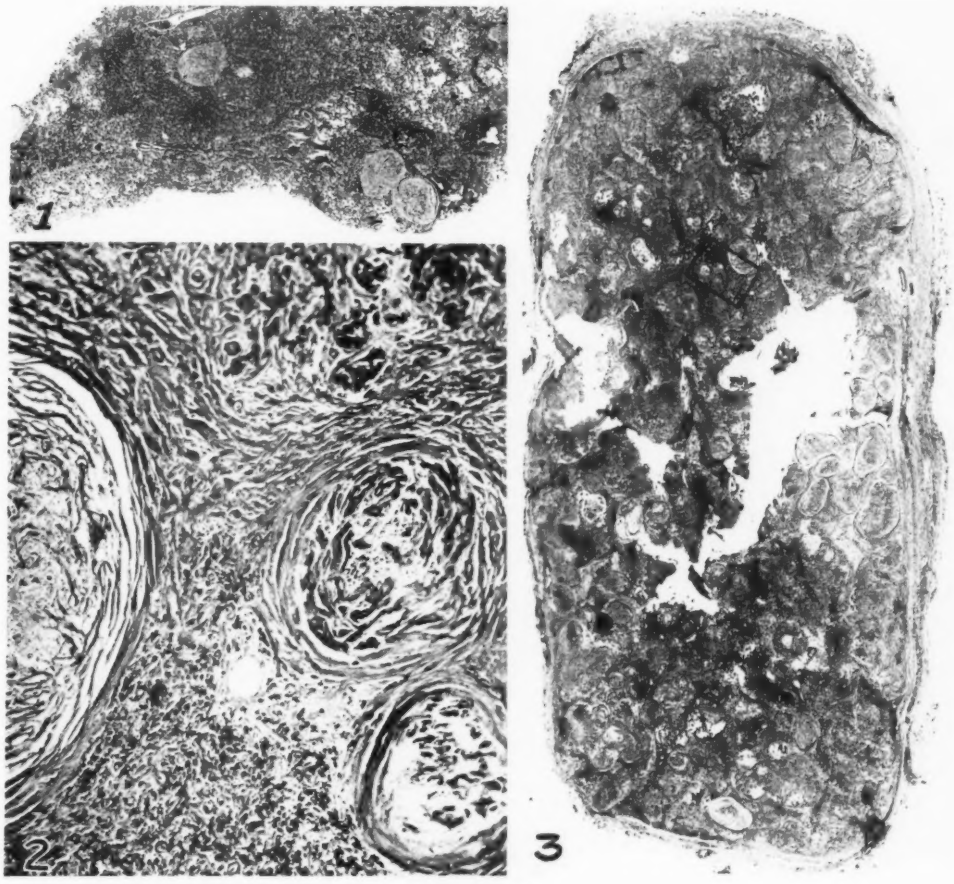


PLATE VII

FIGS. 1 AND 3. Photomicrographs of lung parenchyma and lymph nodes of specimen shown in plate VI, showing characteristic silicotic whorls. × 6

FIG. 2. An enlargement of a region of figure 3 showing whorls undergoing early caseation. × 96

No. 29 was a seventy-nine year old housewife whose lung revealed an ancient primary lesion and a definite reinfection. Histological study revealed some metastatic cancer cells in the region of the hilum, and a culture made of the tissue around tubercle 3A grew one colony of tubercle bacilli. The tissue was treated in alcohol which may have interfered with the growth of tubercle bacilli.

No. 30 was a sixty-five year old white female whose lung showed two tubercles of great age in the left upper lobe, which on culture were negative, and a young (less than one year)

reinfection lesion in the left base about 3 mm. in diameter. The tubercle was negative but the tissue around produced a 4-plus growth. It was highly probable that the bacilli were not confined to the tissues around the lesion but that the trimming and alcohol treatment eliminated all living bacilli from the lesion.

No. 62 was a thirty-seven year old Oriental male with tuberculous spinal caries. There were three positive reinfection lesions of less than a year's age in the lungs. The tissue around tubercle No. 3—a 3 mm. lesion—was 4-plus positive, and from the tubercle itself only one colony developed. The alcohol treatment may have destroyed most of the bacilli, as in the preceding case. Because of these observations it was decided to discontinue alcohol treatment.

TABLE 5

An analysis of the results of culture of single specimens on the basis of the source and wherever possible the age characteristics

CHARACTER OF MATERIAL	ALL TUBERCLES WITH ESTIMATED AGES IN YEARS EXPRESSED IN TERMS OF PRIMARY LESIONS								TISSUE AROUND TUBERCLES	Ca TISSUES	SILICOTIC TUBERCLES	LOST SECTIONS	UNCLASSIFIABLE AND MISCELLANEOUS TUBERCLES	TOTAL	PER CENT
	1	2	3	4–5	6–7	8–10	11–15	16+							
+	2	3	4	6	8	13	10	0	11	3	20	1	2	83	11.05
−	5	4	10	31	24	92	149	127	89	4	61	15	57*	668	88.95
Total.....	7	7	14	37	32	105	159	127	100	7	81	16	59	751	
Per cent +....	28.6	42.9	28.6	16.7	25.0	12.1	6.3	0	11.0	42.9	24.9	6.2	3.8		

* Included the culture of two chondromata, a lipoma and a phlebolith.
Two cultures were contaminated.

No. 87 was a thirty-eight year old female with an overflowing type of lesion from which 12 colonies of bacilli were grown, while the tissue around produced only 2 colonies. Tubercle No. 2, thought to be the lymph node complex of No. 1, was well healed. No bacilli were grown from this lesion, but 3 colonies were grown from the tissue around it. It seemed probable that the bacilli were carried down to the periphery of No. 2 from No. 1 tubercle.

No. 115 was a fifty-eight year old white male laborer with four eight to twelve year type negative lesions on the left and three scattered negative lesions on the right. One lesion (No. 7) was of a silicotic type of fibrosis. From the tissue around it were grown 21 colonies of tubercle bacilli.

No. 143 has been described before. It was a twenty-three year old female Negro with a huge slowly progressive primary lesion in the right base extending up to the hilum. There were lesions of all ages from a fifteen-year type down to young lesions. Tubercle No. 1, an old disintegrating type, was negative, but from the surrounding tissue 36 colonies grew; from lesion 2b, a new process, 38 colonies were grown, but in the surrounding tissue there were only 2 colonies.

No. 170 was a thirty-seven year old male with a large multiple tuberculous complex in the right upper lobe and hilum. From tubercle 1a, at the hilum—a ten-year type—9 colonies grew; 1b nearby was negative. Tubercle 2, in the right upper lobe, produced one colony and from the tissue around it, 3 colonies grew.

In *No. 219* a growth of one colony was obtained from lung tissue far removed from the tubercles, whereas the tubercles were all negative.

No. 225, presented previously, had an extensive progressive process ranging in ages of lesions from twelve years down to recent age types, consisting of monocytic cell infiltration and fibroblasts. Many of the tubercles were 4-plus positive, but only 2 colonies grew from the tissue around tubercle 3.

In *No. 246*, the tissue around tubercle 8a was positive, but the whole region was involved in a fibroid mass around a central core of fibrocaseous tuberculosis.

No. 255, also previously discussed, was a seventy-eight year old male with ten to fifteen year type lesions extending from the right base to the hilum. The lesions towards the hilum produced 4-plus culture growths. The parenchymal lesion was negative but the tissue around produced a few colonies. All the lymph node lesions were positive. The probability is that there had been a new reinfection with bacilli scattered all the way up to the hilum. The local (parenchymal) lesion was no doubt present, but not found.

Of the 11 specimens of tissue around the tubercles, tubercle bacilli in all but 3 (115, 219 and 255) could be accounted for on the basis of infected lesions in the vicinity.

On the other hand, there were 89 wholly negative attempts to grow colonies from perifocal tissue, in spite of the fact that 9 were taken from tubercles having positive findings. Of a total of 21 positive cases studied for growth in the perifocal tissue, there was a positive result in the tissue alone in 3, a negative result in 9 where the tubercle was positive, and a positive result in 9 with positive tubercles. Usually there were many more colonies in the "tubercle cultures" than in the "tissue cultures." The impression was gained that, in all but a small minority, bacilli emanate from tuberculous lesions themselves and not from the lung tissues. The phenomenon is better understood when the pathogenesis of the healing process is understood. Lesions that retain bacilli usually become sterilized from the centre outwards. In the outer part of the capsule bacilli may live for long intervals and actually cause an extension or overflow of the lesion. When the tubercles are trimmed too closely the tissues bearing tubercle bacilli are sometimes removed. Later studies on uninvolved lung tissue also confirm this result. While it must be admitted that exogenous infection may localize bacilli in lung tissue without any signs of reaction, especially in old fibroid tissue (without reactivity), this work has shown that in our locality it is the exception and not the rule for bacilli to be thus found in tissues.

Table 5 also shows the findings in a few cancer metastases, in silicotic types of lesions and in a few miscellaneous groups which will be discussed later.

Outside of the cases having one or more silicotic lesions, the bacilli disappeared

from tubercles rather rapidly and at a regular rate. Although a few cases seemed to show activity in tubercles for more than ten years, the evidence indicated that most enclosed lesions did not retain living bacilli long after two years, and many became sterile after one year. The apparent persistence of the bacilli usually accompanied the "spreading," "overflowing," or otherwise progressing character of the lesions. Sometimes the activity seemed to be confined to only one tubercle and even only a small part of one tubercle. In such cases the bacilli appeared to pass out of the tubercles to lie in the outer capsule or in the tissue beyond, ultimately to form new colonies which usually tend to become encapsulated.

Table 5, showing the relation of age to positive findings, suggests the nature of pathological changes taking place in the lesions. Accordingly, the whole series was grouped as far as possible on the basis of type of lesion—primary, reinfection, etc., and whether there were acceptable signs of activity present. There were several subgroups of each. A preliminary analysis on this basis will follow.

The lesions were grouped into primary and reinfection with several subgroups each. There were 159 primary lesions in which the primary characteristics were classical. The primary lesions were divided into two groups and for the want of better ones were designated by new terms—"unaltered" and "altered." The unaltered type consists of primary lesions which have not undergone any progression that could be observed by microscopical examination; the altered lesions are those in which some progression has taken place, such as epithelioid tubercles or monocytes in or around the capsule. There were 140 cases of the "unaltered" primary lesions, 6 of which were occult and 134 healed. In 5 of the "occult" or "unhealed" cases the lesions revealed the presence of living bacilli. A sixth case (No. 80 in the series) was a forty-two year old white male porter with avitaminosis, delirium tremens and bronchitis, among many other conditions. There was a young primary infection of one and one-half to three year age type in the left base. Although no bacilli were found, the presence of giant cells and monocytes in the capsule indicated activity. No older lesions were found. It must be admitted that this type of lesion in a man forty-two years old may well be considered a reinfection complex, but it seems equally plausible that it may be primary, since no old lesions were found. Similar lesions have been formerly called "essentially" primary. They may be better termed "apparently" primary. In the remaining cases the lesions appeared to be healed on the basis of criteria available at the time of examination.

The next subgroup consists of 18 primary lesions in which one or more were undergoing or had undergone some progression. As stated above they were designated "altered" primary lesions and were further divided into three groups, namely, slowly progressive (5 cases—2 active and 3 healed) and rapidly progressive parenchymal lesions (2 cases—one active and one healed) and progressive lymph node lesions (5 cases—3 active and 2 healed).

The more slowly progressive type is well illustrated by case No. 117 (plate II).

In the group of rapidly progressive lesions, No. 225 has already been described and included in the group designated "obvious" tuberculosis above and in table 2.

A typical occult type is *No. 85*, that of a forty-four year old white housewife who had died of multiple sclerosis. There was a 3-plus Kahn reaction. There was a cluster of primary calcified lesions in the left apex with four to five lymph node lesions towards and in the left hilum. The ages were considered to be eight to twelve years. Two lesions of the cluster in the apex had a recent perifocal spread around the older calcified centres. One was on the mediastinal border and one out towards the subapical region. There was a recent reinfection type lesion in the right subapical region composed of a wedge-shaped infiltrate. Acid-fast bacilli and positive cultures were obtained from the lesion in the left subapical region. The infiltrate in the right was overlooked at the time of the examination of the lungs and was, therefore, not cultured.

A group with progressive lymph node lesions is represented by No. 143, already presented for the "obvious" tuberculosis. The lesions in this group are characterized by a slow extension within individual nodes or in separate nodes which are involved in progressive sequence along the lymph channels.

An "occult" type is *No. 145*, that of a forty-five year old white housewife dead of cancer of the sigmoid. There was a cluster of calcified lesions in the right base and hilum of a six to seven year age type. In the hilum were larger soft lymph node lesions of recent formation. Positive cultures were obtained from the hilum nodes.

Another principal grouping was made of cases having lesions wherein there were "reinfection" lesions in the presence of an old primary complex or at least other older tuberculous lesions.

The whole group of lesions in which a reinfection seemed to be clearly evident included 50 cases. There were 15 uncomplicated reinfection lesions (12 active and 3 healed); 10 cases with lesions having a second lymph node component or "reinfection complex" (5 active and 5 healed); and 25 lesions with some form of silicosis or tissue resembling silicotic fibrosis. It is probable that at least 24 were tuberculous or had at one time been tuberculous. Four cases were definitely silicotic with three first and one second-stage involvement. One case (No. 178) had a first-stage silicosis but there were no tubercle bacilli found and the silicotic tissue was not sufficiently altered to justify any suspicion that tuberculosis had ever been present.

A few illustrations of each type will be given or referred to if they have already been given.

No. 54, already given, is an example of a typical reinfection type. There was an old primary complex in each lower lobe and hilum nodes and a more recent reinfection in both apices. Whether the apical lesions are due to bronchogenic reinfection or to a haematogenous seeding from the older lung lesion may be questioned. The evidence in this case, however, seems to point more to a bronchogenic seeding.

Perhaps a more interesting, yet more uncertain group, is the "reinfection complex" type of Schürmann (15) and Terplan (60). There were at least 4 having active lesions considered to be of this type.

Case No. 8 is a good example, where there was a small bony calcified complex and an extensive recent process involving lung parenchyma and lymph nodes with no lesions of an

intermediate age character, indicating an old completely healed process with a recent new infection.

The silicotic cases included two groups; one group of 21 cases with only a few whorls of typical lesions but not extensive enough to class as silicosis, illustrated by cases 29 and 115; another group with definite silicosis illustrated by cases 169 and 293.

There were 3 definitely unclassifiable cases in which the type of lesion could not be determined at all and are excluded from the preceding analysis. Eighteen others were more or less doubtful but they also contained definite primary or reinfection lesions so that they have been included in the preceding analysis. For example, there were cases having innumerable lesions the origins of which were uncertain. There were many cases having fibroid lesions without calcification and many cases of fibroid pleura, both of which will not receive detailed attention until the main series is reported.

By estimating all the "active" lesions, it was found that there were nearly twice as many reinfections as primary lesions. This ratio is to be expected because of the older age groups which predominated and because the silicosis cases help to swell the number of reinfections. The 44 cases in which activity of some sort was determined included 16 primary lesions with 28 deemed to be reinfection.

As in silicosis, the phenomenon of "dormant" bacilli seemed to exist in cancer tissue. Of 7 cases in which cancer tissue was found, 3 (42.9 per cent) were positive for tubercle bacilli on culture. There is perhaps no tubercle-forming tissue in the cancer tissue. It is a minor observation worth further study.

An interesting collateral observation was noted in cases having signs of generalization in which no living bacilli were found, in spite of the fact that 2 cases had many lesions of two years or less in age, and 2 more were less than four years. In these 4 cases, giant cells were present in many of the tuberculous lesions, but neither positive culture nor animal inoculation was obtained in spite of the fact that from 10 to 20 lesions were studied from each, from which a total of 10 guinea pigs and 242 culture bottles were inoculated. It suggests that mild generalization with recovery is not conducive for the viability of tubercle bacilli in the body.

Another finding of special interest to roentgenologists was that there were 4.3 per cent of cases having nontuberculous calcified focal lesions, not including calcified bronchial cartilages. Among these were 4 chondromata, 2 osteomata (which may have been old tubercles), one calcified lipoma, one fibroma, one phlebolith and one case of ossified fibrous tissue in an old fibroid apex. The last lesion showed ossified blood vessel walls and ossified hyaline connective tissue.

Of the chronic nontuberculous conditions, especially important from the standpoint of differential diagnosis of tuberculosis, there were 16 (5.3 per cent) strictly fibroid apical caps (Anders); 10 cases of bullous emphysema, of which 4 approached cystic proportions; one azygos lobe and the other nontuberculous conditions and cancer metastases mentioned above.

DISCUSSION

Despite many new facts, this study is unfortunately limited with respect to new criteria for the detection of early tuberculosis during life. Clinicians must continue to investigate every clue, roentgenologists must still look for the small, soft flecks and infiltrates or varying types of calcified lesions, and bacteriologists must search for bacilli as they have heretofore. But it is hoped the new data will enhance the efficiency of the methods already in use and give a better understanding of the disease.

Among the new or partly new features shown on roentgenograms and verified by pathological and bacteriological examination, special mention may be made of the type of lesions undergoing apico-caudal progression with decreasing density of the lesions away from the apex and an increase in their size and irregularity caudalward. This is particularly true regarding the arc from the apex to the base of the upper lobe. The same extension may also exist in other lesions below the apex particularly in the subapical region. The differences in age may not always be certain but the softness of the lesion, the size, the fuzziness of its borders and cloudy appearance speak for relative recency in development.

Another unstable type of lesion which is subject to refined roentgenological interpretation is the round infiltrate (Albert) with a "soft" border often poorly defined. The "soft" border is a definite sign of relatively recent activity. This type of lesion has been described before as "overflowing" or one with peripheral progression (59). Not less suspicious of activity are the ones with old dense calcifications in the lower lung fields and soft or semidense lesions in one or both apices. As reported earlier by Opie (14), and well demonstrated in this study, these apical lesions are more prone to harbor living bacilli than calcified lesions in the base (see plate I). They are generally interpreted as reinfection type of exogenous origin but many, as Lydtin (61) has pointed out, are haematogenous. They may be either bronchogenic or haematogenous. Finally, all soft or cloudy masses in proximity to more dense lesions must continue to be viewed with as grave apprehension as they are at present.

With regard to negative bacteriological findings, it may be said that there are features about which more certain statements can be made. First may be mentioned the sharp dense lesions in clear or emphysematous lung tissue. The shadows may be round, irregular, or fenestrated and need not be limited in size or number. Of course the larger the original lesion, the more likely is the possibility of its retaining living bacilli (see No. 143, plate III), but the "setting" of the lesion is of even much more importance. If there is no "cloudy" tissue around, if the lesion stands out "like Mars at perihelion," and if the hilar lymph nodes are not prominent, it is probably healed.

There has been much discussion of calcified lesions since World War II began and the difficulties of the Army and Navy in setting up standards in a short period of time can well be appreciated. The recommendations, while perhaps not always right, were on the side of safety, especially with the knowledge of the many and varied conditions under which the examinations were to be carried out

and the hazards to which the inductees were to be subjected. Although all tuberculosis does not necessarily spread from the large lesions, the odds are in favor of such a procedure because of the mass of tissue involved, as well as the natural lag in the healing of such lesions. Chadwick and Zacks (62), for example, reported that five times as many cases of clinical disease developed from the large calcified lesions as in cases with positive tuberculin reaction and no calcified lesions.

On the other hand, it would be a mistake to overlook the small, soft lesions, especially the adult primary types which frequently develop into rapidly progressing disease. *Any soft lesion is dangerous irrespective of its size.* The important feature is the presence of living and virulent tubercle bacilli with a thin capsule or with no barrier but cells between them and the lung canals. There is, therefore, no absolute rule to apply to every case.

The lymph node lesions offer even greater problems than those in the parenchyma. With the exception of the large, bulging hilum there is no certain way to tell active from healed lesions in the hilar nodes of the lung. If a lymph node lesion protrudes beyond the central density and appears poorly calcified or merely hyperplastic, they may at once be considered progressive or only in the process of healing, but certainly not healed. Although we have not explored the possibilities of newer roentgenological methods, the planograph and multiangular views with differences in exposure may help to explore the "unknown" hilum region so far as activity of tuberculosis is concerned. It would seem that such a study is indicated.

With regard to stringy fibrosis, only the most general suggestions are warranted. Bulky lesions, even if they appear only as strings, may still contain living bacilli and are subject to suspicion. Completely healed fibrous lesions are prone to be so thin and so thoroughly masked by emphysema that they usually arouse little or no suspicion. It is surprising how little may be left in the form of scar tissue of what may once have been an extensive lesion. In fact, many of them may not be even seen in the emphysema which is so prone to result from the healing process. On the other hand, it must be pointed out that emphysema progressing to a debilitating cystic condition may not be a result of tuberculosis at all.

Only a similar generalization can be given for the various types of pleural lesions. The apical (Anders') caps are, with few exceptions, either benign or nontuberculous. As pointed out by Kayser-Petersen (63), Braeuning (4) and others, the apical tuberculous lesions are likely to be more benign than lesions developing lower down. As a rule, pleural lesions if associated with fluid or a fluid residue, must be considered potentially dangerous.

Of all the potential means of detecting activity, it would seem that the tuberculin reaction might offer assistance, but has thus far not been thoroughly exploited. By comparing this study to the tuberculin studies, it can be generally stated that only one in 4 or 5 cases with a positive tuberculin reaction will have positive cultures of tubercle bacilli. In the others, the bacilli are dead and the tuberculin reaction is probably only diminishing to extinction, as suggested by

Long (64). It is possible that only the cases having the strongest reactions are the most recent and hence are the most prone to contain living bacilli. Of course, allowances must be made for desensitization as the active disease develops. The field of immunology seems to offer a ray of hope for aid in the detection of activity.

It was further observed that even in a heavily infected district there are many cases in late adult life without demonstrable tuberculosis. While a few may never have been infected, the probability is that the majority of the lesions of this series have either been too small to be seen, have been resorbed, or there may have extrapulmonary lesions which would perhaps be more numerous in the late decades of our series because these people lived in a time when bovine bacilli were more prevalent. Even to-day in certain districts (see Blacklock, (65)) bovine gastrointestinal infection may account for over a third of tuberculous infections. In spite of this possibility, however, the majority of cases in our series appear to show that reinfections have occurred and not only reveal lesions in the lungs on X-ray films, but also have reëstablished the positive tuberculin reaction. Although it must be admitted that the knowledge of the pathology of the tuberculous lesions cannot be used for antemortem diagnosis, it can, however, be applied indirectly to lesions visible on the X-ray film, which in turn must continue to afford the guide in living patients. A general rule is that regressive lesions begin to extinguish tubercle bacilli very soon after encapsulation (within three to six months) and by the time they show calcification (in one to two years) the majority of bacilli are dead. A few bacilli survive for a period of five years after tubercle formation with an exceptional case having living bacilli after ten years. Not infrequently, a new lesion can be followed during the process of development by fluoroscope or X-ray film and checked by a tuberculin reaction. As the months and years pass, their danger rapidly decreases. Many times, the age of a lesion observed on X-ray films may be approximated and its relative safety predicted, especially if the surrounding tissues reveal a disappearance and not an extension of activity. Most of the persistent infections are perhaps due to perifocal reimplantations, which are prone to reveal themselves as new, soft shadows.

Enough people retain bacilli to account for a large number of our active cases of tuberculosis that mature like any crop of biological units a few months or years after seeding.

Nothing has been found that will account for the persistence of bacilli in any particular lesion, but it is probably the old question of "seed" and "soil." Of course some are obviously due to a lowered resistance, poor "environment" (Krause), or to constitutional weakness of the host, but it would seem also equally probable that the exacerbations are due to an increase in virulence or to an adaptability of a newly generated strain of bacilli to its surroundings. Such a hypothesis is in keeping with sound biological principles.

A valuable clue regarding the development of silico-tuberculosis has become apparent, although only preliminary observations can be expected at this time. In none of the lesions resembling silicosis could there be found any signs of recent tuberculous activity. The bacilli appear to remain in the old nodes and produce a slight caseation and a peripheral or "egg shell" calcification (50), but no cellular

reaction. Perhaps the defense mechanism may have become exhausted within the nodules, or the conditions may be such that the bacilli can exist, but not multiply (until later in the disease). While the bacilli may survive longer in the silicotic lesions, there does not appear to be any more active tuberculosis in these cases than in those without silicosis. In fact, there was a much lower percentage of positive findings in the minimal silicotic types than in those showing progressive tuberculous lesions without silicosis. However, when the bacilli become overwhelming or the host's vital reserves become depleted, the bacilli spread into the uninvolved tissues and the well known terminal tuberculosis appears. It would seem, therefore, that a case of silico-tuberculosis may live for long intervals if the critical period of exacerbation can be guarded against.

From a composite analysis of the present study, the work of others and generally accepted facts, the various phases of tuberculous infections may be reconstructed about as follows: The bacilli enter the body usually by way of the lungs with only about 10 per cent of infections taking place in the intestines and upper respiratory tract. This ratio changes, however, in places where bovine bacilli are prevalent. Where there is no control of cattle herds for tuberculosis the extrapulmonary lesions may rise to 35 and 40 per cent.

In a period varying from three weeks to three months the cells of the body become sensitized to tuberculin depending largely upon the size of dosage. The sensitization thus acquired persists for a period of five years to life, depending on the healing process. In healing, the sensitivity tends gradually to diminish. In the process of healing, tuberculin sensitivity probably persists for a period of three or more years after the bacilli are dead. Like any other immune reaction there is a variable period in which it persists after the absorption of antigen. If the infection survives, the sensitivity may continue with varying degrees of desensitization or until overwhelming disease results in complete anergy. It is furthermore possible for reinfections to cause a resensitization which varies with the prevalence of open disease in the respective localities.

There is a strong tendency for most tuberculous lesions to heal, especially those caused by moderate or small dosages. In such lesions tubercle bacilli tend to disappear soon after the lesion is formed. The majority are dead within three years and there is a rapid diminution of the remainder up to ten years, with none surviving after fifteen years. A small percentage of all infected cases progress into disease in an average period of about ten years, but such cases are either terminated by death in about 3.5 years (66) or are healed by medical or surgical treatment. In former times, however, a larger number remained alive for longer intervals as chronic open cases.

Within a period of a few weeks after infection the lesions may become visible on roentgenograms as soft, round, cloudy, flaky densities, varying in size from the point of visibility to large masses, as described by various workers, including Rigler and Exner (67), Braeuning (4) and others. The majority of small lesions, including lymph node complexes, are not visible on the ordinary antero-posterior views, although most of the significant lesions are perhaps visible at some stage of the process. Fibrous tissue encapsulation begins within a few months and

increases for six to eight years. Calcification begins within one to two years and progresses for eight to ten years, when resorption occurs and continues for life with some of the smaller foci completely disappearing. Due to certain unknown causes a small percentage of lesions retain living bacilli which may spread rapidly, slowly, or lie dormant for long intervals, sooner or later to end in the variegated disease entity known as pulmonary tuberculosis.

There were numerous other interesting observations made in the study but descriptions and discussions of them can better afford to wait for the analysis of the completed series.

SUMMARY

The study involves a report of lesions in the lungs and related lymph nodes of 300 patients dead of conditions other than tuberculosis in several large general hospitals and from the coroner's laboratory of the City of Chicago.

There were 254 (84.7 per cent) cases having lesions with densities deemed to be calcified tubercles, leaving 46 (15.3 per cent) negative. There were 42 cases (14 per cent) with lesions too small for satisfactory study. Together with the preceding group it makes 88 (29.3 per cent) of unstudied cases. The remaining 212 cases (70.7 per cent) were dissected and the dense lesions removed and studied.

Of the 212 cases studied, 5 cases (2.4 per cent) had a history or clinical findings of tuberculosis but no tubercle bacilli were ever demonstrated during life. Four cases (1.9 per cent) were negative throughout, but positive on gross and microscopical pathological examination. The two groups together made a total of 9 cases (4.3 per cent) of "obvious" or frank tuberculosis but without the demonstration of tubercle bacilli ever having been made before death.

The remaining 203 cases (95.8 per cent) of "occult" and healed tuberculosis consisted of 168 with entirely healed lesions so far as our studies could reveal and 35 (16.5 per cent) "occult" lesions. Of the latter, 29 (13.7 per cent) were positive on culture and 6 others were considered positive because of strong evidence afforded by acid-fast stain, animal inoculation and pathological findings, even though the cultures were negative.

The grouping of the 212 lesions on the basis of primary or reinfection gave 159 primary lesions with 50 having definite reinfection lesions and 3 unclassifiable.

Of the primary lesions, 140 were typical or "unaltered" without any microscopical evidence of progression. Six revealed evidence of residual activity and 134 were entirely healed. Eighteen cases were found to have microscopical evidence of peripheral progression in the capsule or outside of the tubercles in either the lung parenchyma or lymph nodes.

The reinfection lesions consisted of three groups: 15 isolated lesions; 10 "reinfection complexes;" and 25 complicated with silicosis or a fibrosis resembling silicosis.

Approximately half of all the altered types of lesions possessed some form of activity. Of the 44 active cases, 16 were apparently primary and 28 were thought to be some type of reinfection lesions.

Seven per cent of the cases revealed calcifications in the first decade of life, 55 per cent in the second, 80.4 per cent in the third, with the number approaching 100 per cent in the later decades.

Positive cultures were obtained on 36 cases (17.0 per cent) of the 212 cases in which detailed studies were made. Twenty cases (9.4 per cent) were positive on cultures alone. Of the same group, only 16 cases (7.6 per cent) showed acid-fast bacilli on smears, which were definitely significant. Two cases (2 per cent) were strongly positive on smear but negative by culture. There were 9 cases (4.3 per cent) in which acid-fast bacilli were found, but usually a lone bacillus was reported, and without positive cultures or other signs of activity they were considered as saprophytes or "dead" bacilli and were disregarded. Three cases were considered positive by pathological means only because of signs of activity in and around the capsule. Two cases were included which were active extrapulmonary tuberculosis. All methods together revealed 44 cases (20.8 per cent) which had activity.

Direct smears were rarely positive when there were less than 15 colonies on one of the culture bottles. In a few cases there was a wide variation in the results on different media used, but a final statement will not be given until the whole series is complete.

Tubercle bacilli were prone to live in or near the outer capsules of tubercles, as shown in microscopical tubercles, monocytes and giant cells in close proximity to the main lesions.

Positive cultures made from tissues entirely outside of the tubercles were rare and appeared to decrease as the distance increased from active tubercles.

Living bacilli were prone to exist in the young type of lesion. When the cases were arranged according to the ages of the youngest lesions, there was a positive finding of 80 per cent in tubercles less than one year of age and a gradual drop to 0 per cent at the end of fifteen years.

Of 8 cases having a few whorls or palisades of fibrosis and pigment resembling silicosis, one (12.5 per cent) was positive; of 21 having several fibrotic whorls, 5 were positive (23.8 per cent); and of 4 first- and second-stage cases of silicosis, 3 were positive (75 per cent). A few whorls of fibrous tissue resembling silicotic fibrosis do not seem to increase the incidence of living tubercle bacilli in tuberculous lesions.

In 7 cases where partial generalization occurred, no bacilli could be found in spite of the fact that in 4 of the cases the lesions were less than four years and 2 had lesions appearing of less than two years duration. It would suggest that wide-spread lesions develop a higher degree of immunity against the bacilli.

There were 13 cases (4.3 per cent) which showed "densely calcified" or "partially calcified" lesions on the roentgenograms which were not tubercles or silicotic nodules but were associated with the more common calcified lesions. They were chondromata and osteomata, with one lipoma, one fibroma and one phlebolith.

There were 10 cases of bullous emphysema, 4 of which were cystic in character.

SUMARIO

Este estudio versa sobre las lesiones en los pulmones y ganglios linfáticos regionales de 300 enfermos que murieron de estados distintos de la tuberculosis en varios grandes hospitales generales o fueron estudiados en el laboratorio médicoforense de la ciudad de Chicago.

En 254 casos (84.7 por ciento) había lesiones con sombras consideradas como tubérculos calcificados, dejando 46 (15.3 por ciento) negativos. Hubo 42 casos (14 por ciento) con lesiones demasiado pequeñas para estudio satisfactorio, los cuales junto con el grupo anterior representan 88 casos no estudiados (29.3 por ciento). Los otros 212 casos (70.7 por ciento) fueron disecados y las lesiones espesadas resecadas y estudiadas.

De los 212 casos estudiados en 5 (2.4 por ciento) había historia o hallazgos clínicos de tuberculosis, pero jamás se encontraron en vida bacilos tuberculosos. Cuatro casos (1.9 por ciento) fueron negativos constantemente, pero positivos al hacerse el examen patológico macro y microscópico. Los dos grupos unidos forman un total de 9 casos (4.3 por ciento) de tuberculosis declarada o manifiesta, en los que, sin embargo, jamás se encontraron bacilos tuberculosos antes de la muerte.

Los otros 203 casos (95.8 por ciento) de tuberculosis "oculta" y cicatrizada comprendían 168 con lesiones absolutamente cicatrizadas en lo que pudo revelar este estudio, y 35 (16.5 por ciento) con lesiones "ocultas." De las últimas, 29 (13.7 por ciento) resultaron positivas al hacer cultivos y 6 se consideraron también positivas debido a datos poderosos aportados por la coloración ácido-resistente, la inoculación en animales, y los hallazgos patológicos, aunque los cultivos fueron negativos.

La clasificación de las 212 lesiones, a base de lesión primaria o reinfección, rindió 159 lesiones primarias con 50 lesiones precisas de reinfección y tres inclasificables.

De las lesiones primarias, 140 eran típicas o "inalteradas" sin signos microscópicos de agravación. Seis revelaron signos de actividad residual y 134 estaban absolutamente cicatrizadas. En 18 casos se descubrieron signos microscópicos de avance periférico en la cápsula o fuera de los tubérculos, bien en el parénquima o en los ganglios linfáticos.

Las lesiones de reinfección comprendían tres grupos: 15 lesiones aisladas; 10 "complejos de reinfección" y 25 complicadas con silicosis o con fibrosis silicoidea.

Aproximadamente la mitad de todas las lesiones de tipo alterado mostraron alguna clase de actividad. De los 44 casos activos, 16 eran aparentemente primarios y 28 pasaron por ser lesiones de reinfección de algún tipo.

Revelaron en el primer decenio de la vida calcificaciones: 7 por ciento de los casos, 55 por ciento en el segundo, y 80.4 por ciento en el tercero, aproximándose la proporción a 100 por ciento en los decenios subsecuentes.

Obtuviéronse cultivos positivos en 36 casos (17.0 por ciento) de los 212 casos en que se realizaron estudios pormenorizados. Veinte casos (9.4 por ciento) resultaron positivos a base exclusivamente de cultivos. Del mismo grupo sólo

16 casos (7.6 por ciento) revelaron en los frotes bacilos ácido-resistentes que tuvieran importancia bien definida. Dos casos (2 por ciento) fueron intensamente positivos en los frotes, pero negativos conforme a los cultivos. Hubo 9 casos (4.3 por ciento) en que se descubrieron bacilos ácido-resistentes, pero por lo general no había más que un bacilo y de no haber cultivos positivos u otro signo de actividad se consideraron como saprofitos o bacilos "muertos," desatendiéndose. Tres casos se consideraron positivos patológicamente sólo por haber signos de actividad en la cápsula y alrededor de ésta. Dos casos se agregaron por mostrar tuberculosis extrapulmonar activa. Todas las técnicas combinadas revelaron 44 casos (20.8 por ciento) en que había actividad.

Los frotes directos fueron raramente positivos cuando había menos de 15 colonias en uno de los frascos de cultivo. En algunos casos variaron ampliamente los resultados en los distintos medios utilizados pero el informe definitivo se dejará hasta completar toda la serie.

Los bacilos tuberculosos se mostraron inclinados a vivir en las cápsulas externas de los tubérculos o cerca de éstas, según revelaron los tubérculos microscópicos, monocitos y células gigantes próximos a las lesiones principales.

Los cultivos positivos obtenidos de los tejidos absolutamente fuera de los tubérculos fueron raros y parecieron disminuir al aumentar su distancia de los tubérculos activos.

Los bacilos vivos mostraron tendencia a existir en las lesiones de tipo reciente. Al disponer los casos conforme a la antigüedad de las lesiones, resultaron positivos 80 por ciento de los tubérculos de menos de un año con una baja gradual a 0 por ciento al cabo de 15 años.

De 8 casos en que había algunos verticilos o empalizadas de fibrosis o pigmentos parecidos a silicosis, uno (12.5 por ciento) fué positivo; de 21 en que había varios verticilos fibróticos, 5 fueron positivos (23.8 por ciento); y de 4 en el primero y segundo período de la silicosis, 3 (75 por ciento). Algunos verticilos de tejido fibroso que semejan fibrosis silicótica no acrecientan aparentemente la frecuencia de los bacilos tuberculosos vivos en las lesiones tuberculosas.

En 7 casos en que había tenido lugar una generalización parcial no pudieron encontrarse bacilos a pesar de que en 4 las lesiones tenían menos de 4 años y 2 mostraban lesiones que parecían de menos de 2 años de duración. Esto indicaría que las lesiones esparcidas producen una inmunidad mayor a los bacilos.

Hubo 13 casos (4.3 por ciento) que revelaron en los roentgenogramas lesiones "densamente calcificadas" o "parcialmente calcificadas," que no eran tubérculos o nódulos silicóticos, pero estaban asociados a las lesiones calcificadas más comunes, siendo condromas y osteomas con un lipoma, un fibroma y un flebolito.

Hubo 10 casos de enfisema buloso, 4 de ellos de naturaleza quística.

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THE ACTION OF ARTIFICIAL GASTRIC JUICE AND DUODENAL SECRETIONS ON TUBERCLE BACILLI[1]

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In a recent publication (9), the authors have shown that, in 8.5 per cent of guinea pigs inoculated with sediment from the gastric washings of 219 adolescent contact cases, acid-fast bacilli could be demonstrated in pressure smears from the spleen. Cultures and secondary inoculations from this material, as well as from the gastric washings, were entirely negative. Failure to obtain positive results could be explained on the ground that these bacilli were not tubercle bacilli or that the gastric secretion had killed them. This latter possibility, although investigated previously, has been reëxamined.

Fifty years ago Straus and Wurtz (1) demonstrated that gastric juice from the dog would not destroy tubercle bacilli following exposure of from one to six hours. When the organisms were subjected to the action of the secretion from eight to twelve hours, only local lesions followed animal inoculation. If eighteen to thirty-six hours was the test period, no lesions were produced. Frank and Fischer (2) obtained somewhat simular results, showing that the tubercle bacillus was able to survive intimate contact with gastric juice for at least as long as the ordinary digestive period. Calmette (2) laid great emphasis on the variation in virulence of the bacillus in the production of experimental lesions in the digestive tract; believing that this fact explained the lack of uniformity of results, rather than any modification of the organism by the action of the digestive secretions. Fenwick and Pasquier (2) noted that in early tuberculosis hyperchlorhydria was present in 50 to 60 per cent of the cases, but the latter observer also found hypochlorhydria in 76 per cent of the advanced cases. What connection this finding has in relation to the prevalence of tuberculous colitis may be debatable.

METHODS

Pure cultures of tubercle bacilli were obtained from the sputum of 10 patients suffering with chronic pulmonary tuberculosis. Corper's (4) and Steenken's (5) methods were used to isolate bacilli. The 10 strains were shown to be virulent and this characteristic was maintained unchanged throughout this work.

In order to have a standard material which could be utilized over a considerable period of time, artificial gastric juice of the following composition was used.

Pepsin 5 gram	
Hydrochloric acid	6.0 cc.
Lactic acid	2.0 cc.
Water to	1,000 cc. (pH 1.4)

The duodenal contents[2] were obtained from healthy young adults by the use of the duodenal tube. This was inserted into a fasting stomach and, under

[1] From the Boston Health Department, Boston, Massachusetts.

[2] Composition: bile, pancreatic secretion, duodenal secretion.

the fluoroscope, carried into the duodenum. When the amount of fluid in the upper bowel was limited in quantity, a solution of epsom salt was injected through the tube. This method allowed the collection of 50 to 100 cc. of duodenal contents with which to work. The fluid was then filtered through a Berkefeld candle and used within two or three hours.

Suspensions of tubercle bacilli were made up as follows (6): A small portion of moist culture, weighed on sterile tinfoil, was placed in a mortar and ground with five drops of a sterile 5 per cent solution of taurocholate. During twenty minutes of grinding, sodium phosphate buffer solution was slowly added up to 3 cc. and the material allowed to settle for thirty minutes. One cc. of the supernatant fluid was then added to a Hopkins tube (3) and if the reading, after one-half hour of centrifugalization, was noted as 0.01 mg., dilutions in a series of one to ten were made, using the original supernatant suspension.

A dilution containing .001 mg. of the bacilli was used in the experiments. The observations of Wilson and Schwabacher (8) indicate that a bacillary mass of this weight contains 1,000,000 bacilli of which "only 5 to 10 per cent . . . are culturable." Suspensions of the 10 cultures of tubercle bacilli were made and 1.0 cc. of the selected suspension was added to 15 cc. of artificial gastric juice and incubated at body temperature for six- and twelve-hour periods. This time of exposure was chosen, as a three-hour interval gave completely positive results. Following incubation, the test mixture was neutralized and the centrifugalized sediment injected into the groin of a guinea pig. At the end of six weeks the animal was autopsied. Macroscopical lesions were noted and pressure smears and cultures were made from the spleen and the lymph nodes. This experiment was repeated three times with each culture. A fourth test was made using filtered doudenal contents alone both in six- and twelve-hour incubation periods. A fifth test consisted of exposing the bacillary suspensions to artificial gastric juice for a six- and a twelve-hour interval, followed after neutralization, by a three-hour exposure to duodenal contents. Animals were then inoculated as before with the sediment of the suspension and, after a six weeks' observation period, were sacrificed.

Of 100 animals which were inoculated in these five series of tests the results of autopsy showed a decreasing diversity of lesions, depending upon the time element, except in the fourth group where the disease was most extensive. The lesions in the six-hour group were about one-half those noted in the three-hour exposure period and the twelve-hour period was nearly 80 per cent less.

The observation of Straus and Wurtz that only local lesions occurred at the site of inoculation following the action of gastric juice in the dog were not duplicated. Allowance should be made, however, for variation in virulence and for the number of organisms injected.

Little or no effect upon the tubercle bacillus, as indicated by the extent of the lesions, was noted following exposure to filtered duodenal contents and it did not enhance the effect of artificial gastric juice when used subsequently to it.

CONCLUSIONS

The effect of the exposure of virulent tubercle bacilli to artificial gastric juice varies with the duration of such exposure.

Filtered human duodenal contents, containing bile and pancreatic juice, do not alter the infectivity of the organism for the guinea pig.

Although large numbers of virulent tubercle bacilli were used to infect this series of animals, in none were the lesions produced massive and on direct smear or culture very few organisms could be recovered.

CONCLUSIONES

El efecto de la exposición de los bacilos tuberculosos virulentos al jugo gástrico artificial varía según la duración de dicha exposición.

Los filtrados del contenido duodenal humano, que contienen bilis y jugo pancréatico, no modifican la infectividad del microbio para el cobayo.

Aunque se utilizaron grandes cantidades de bacilos tuberculosos virulentos para infectar esta serie de animales, en ninguno de éstos fueron las lesiones producidas masivas, y no pudieron descubrirse más que muy pocos gérmenes ya en frote directo o en los cultivos.

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EXPERIMENTAL TUBERCULOUS PLEURAL EFFUSION

The Causal Relationship of Tubercle Bacilli and of Specific and Nonspecific Protein to Its Production

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Clinical and experimental work has been done in studying the reactions of the pleura to irritation and the various inflammations to which it is subject. There has been especial interest in the reactions of the pleura to the tubercle bacillus. The following report deals with the effect on the pleura of tuberculous animals produced by the introduction of both nonspecific and specific tuberculin protein.

LITERATURE

Pinner, Moerke and Saley (12), in an exhaustive report, have described the changes of permeability of the pleura that occur during inflammation. They expressed the belief that inflammation produces an alteration of permeability and based their belief on the fact that constituents of plasma pass very rapidly into the pleural space. In their opinion the inflammatory process increases the permeability of the endothelial linings of the capillaries and the degree of alteration is indicated by the number of haematogenous cells found in the resultant effusions. Such a concept agrees with the clinical findings, in which the largest number of erythrocytes is found in the effusions subsequent to the most acute infections. In the opinion of Pinner, Moerke and Saley, resorption of fluids begins before isotonicity is established between the effusion and the blood plasma, but it becomes rapid when isotonicity is established. On the other hand, they expressed the belief that in the diseased pleura effusions exist for a long period and that the delay of resorption is due, not essentially to chemical constituents of the effusion, but rather to changes that have taken place in permeability of the separating membranes. The response of the normal pleura to irritants is the production of an effusion varying in amount and in cellular constituents with the intensity of the inflammatory changes produced by the irritant and the consequent variation of its permeability.

Higgins and one of us (W. S. L.) (4, 6) observed the effects produced by the introduction of many different particulate inorganic substances into the pleural space of normal animals. The responses varied only in the degree of acuteness of the inflammation produced by the various particulate substances. In each instance the normal pleura became congested and oedematous, and fluid rapidly accumulated. The most severe inflammations resulted in an effusion that contained the largest number of erythrocytes. In all effusions, however, all the cellular constituents of the blood were represented, but not in the same proportion as was found by differential blood counts. There was an excessive number

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of polymorphonuclear cells which immediately exercised their function of phagocytosis and within seventy-two hours degenerated and died. They were succeeded by increasing numbers of mononuclear histiocytic elements, which were equally active as phagocytes and removed cellular debris as well as particles. The mononuclear phagocytes served to heal the damaged pleural surfaces by taking part in the formation of scar tissue.

Likewise, Montgomery and one of us (W. S. L.) (7, 9) observed the effects produced by the introduction of virulent bovine tubercle bacilli into the pleural space of the normal rabbit. The effect was materially different. The bacillus is much less irritating to the pleural tissues than particles of graphite, silica or aluminum oxide of approximately the same size, and produces only an extremely slight inflammatory reaction and a very small and transient effusion. The phagocytic cells are the same, absorption occurs by way of the lymphatic vessels in the same manner whether the particles are organic or inorganic, but healing by scar formation does not occur in rabbits. These animals soon (within fifteen to thirty days) become sensitized and then the effusion occurs. This infection spreads to the lungs and the animals die of acute disseminated tuberculosis. In 1917, Paterson (11) reported a similar experience. He was unable to obtain an immediate pleural reaction or an effusion of demonstrable proportions following the introduction of virulent tubercle bacilli into the pleural spaces of normal animals. When the animals had been sensitized by previous subcutaneous inoculation of slightly virulent bacilli, then an immediate effusion resulted from the subsequent intrapleural inoculation of bacilli.

Following intrapleural injection of virulent bacilli, we permitted our experimental animals to live and found that, although the original small effusion had disappeared in three to four days, it reappeared after approximately two weeks. Then it progressively increased in amount and persisted until the animals died of tuberculosis. The reappearance of the second effusion corresponded to the development of allergy, as expressed by a positive cutaneous reaction to tuberculin. The response of the normal and of the sensitized pleura was of the same inflammatory character, but differed in severity and duration. Paterson found that he could always obtain an effusion if he injected virulent tubercle bacilli into the pleural spaces of animals sensitized by previous subcutaneous inoculation. We have repeated Paterson's experiment and confirmed his findings.

There is, therefore, a more complex reaction when the pleural irritant is an organic substance than when it is inorganic. The development of pleural sensitization or previous pleural sensitization always permits of an inflammatory reaction and an effusion when reinfection takes place. Sensitized pleural tissue responds to reinfection by inflammation, which in turn alters permeability so that there is a sudden passage of plasma constituents into the pleural space. Montgomery and Lemon (7, 9) found that an effusion regularly formed and progressively increased in amount when intrapleural injections of virulent bacilli into sensitized animals were made.

Before proceeding with the introduction of specific and nonspecific protein into the pleural space of sensitized animals, it was necessary to review the changes

that occur when a tuberculin test is made on the tuberculous animal that is known to have its tissues sensitized. Maurice Nicolle (10) evolved the hypothesis of the presence of lysin of tuberculous endotoxin. Calmette (1) said that the tuberculin reaction "results from this 'lytic action' on the part of certain substances contained in the body fluids of subjects affected with the tubercle bacilli upon the tuberculin, and that the lytic action gives rise to the formation of a specific product both toxic and fever-producing." This, in his opinion, is the essential factor in general or local tuberculin reactions. The hypothesis of Nicolle was accepted by Wolff-Eisner (15), as well as by Calmette, and became the basis for the ophthalmic reaction that is known by their names. Calmette said, "If it is true that lysin accumulates or is produced in greater abundance about tuberculous foci in evolution, it explains why these foci, following an injection of tuberculin, become the seat of inflammatory processes so definitely localized."

The work of Stewart (13, 14), of Long, Vorwald and Donaldson (8) and of Krause (5) emphasizes the differentiation of sensitivity. They stated that the allergic reactions produced by secondary contact of the bacillus of tuberculosis or its bacillary products vary greatly. The reaction is always maximal in the diseased organ itself; its severity decreases in organs far removed from the focus of infection and is least in those organs which in themselves do not contain any lesions. The allergic reaction, however, is always an inflammatory one and is characterized by an exudation of serum and the cellular components of the blood.

Calmette showed that in the conjunctival secretion following the introduction of tuberculin there is a marked predominance of polymorphonuclear leucocytes and degenerated epithelioid cells. On biopsy, the conjunctiva is found infiltrated with large mononuclear cells, in almost all of which there is a more or less extensive vacuole, which, in his opinion, represents secretory hyperactivity.

Work by Feldman and Fitch (2, 3) emphasized the resemblance of the lesion produced by tuberculin injected into sensitized tissue to a tuberculous lesion. They recognized that there are oedema and exudation of serum and cellular constituents of the blood, and that in the earliest reaction polymorphonuclear leucocytes predominate. They made the observation that within seventy-two hours the mononuclear cells (which in their opinion are histiocytes) predominate and replace the polymorphonuclear leucocytes which, during that same period, have died. They reported that the histiocytic reactions occur mainly about the adventitious zone of the blood vessels and the tissues about the nerves and, exactly as in tuberculous disease itself, thrombosis of vessels is sometimes observed, consequent on marked endarteritis, proliferation and obstruction resulting from the inflammation.

The pathological changes that have been described by Feldman and Fitch (3) are essentially similar to those resulting from an acute inflammation and the varying responses correspond almost exactly to those that Higgins and one of us (W. S. L.) (4, 6) obtained when we introduced inorganic substances into the pleura of normal animals. When we introduced tubercle bacilli into the normal pleura, the immediate reaction was extremely mild and the effusion very small,

but the cytologic response was identical with both that of the nonspecific particulate matter and that of tuberculin in sensitized tissue.

On the basis of these facts, it seemed probable that specific tuberculin protein would produce an inflammatory reaction accompanied by an effusion when introduced into the pleura of a tuberculous animal whose tissues were known by the intracutaneous reaction to be highly sensitive. This was to be expected because, at necropsy on rabbits selected from the series, the animals were found to be grossly infected. The visceral pleura was elevated by subpleural tubercles and the sensitization reaction was positive. Presumably, also, nonspecific proteins, known to be absorbed without producing an inflammation, would not produce a pleural effusion.

METHODS OF EXPERIMENTATION

Twelve rabbits were inoculated subcutaneously with 0.1 mg. of tubercle bacilli of bovine origin. Fifty days later they were found to give positive reactions to 0.02 cc. of 1:100 dilution of Old Tuberculin. As a control, nonspecific protein as represented by skimmed milk was introduced intrapleurally into 6 of the rabbits. The other 6 were inoculated intrapleurally with potent Old Tuberculin, 3 with 1:100 and 3 with 1:1,000 dilutions.

The animals were killed in pairs at intervals varying from two to twenty-nine days. In not a single instance was there a sign of an effusion and the appearance of both parietal and visceral pleurae was as if nothing had been done. This surprising negative result of the intrapleural inoculation of both specific and nonspecific protein into sensitized animals cannot be explained with any certainty. It seems logical to believe, however, that absorption of fluids might have occurred with such great rapidity that there was insufficient time for an inflammation to result. Higgins and one of us (W. S. L.) (4, 6) found that phenolsulfonphthalein injected into the pleural space could be identified in the urine within seven minutes. If tuberculin is absorbed with equal rapidity, the normal permeability may not be disturbed and therefore no effusion would occur. Such an explanation is not entirely convincing because tuberculin must be absorbed rapidly from the much less sensitive conjunctival tissue, where an inflammation is regularly produced.

In the light of our former experiments (7, 9), however, in which we knew that after ten days fluids occurred, it seems probable that they did so because not all of the bacteria were removed by way of the lymphatic vessels. The bacteria were found to increase in vast numbers and they in turn, acting like particulate foreign bodies, brought about a recurrence of fluid. Tuberculous tissue developed along the course of the draining lymphatic vessels and hindered removal of the effusion. It may be, also, that the fluid in the work mentioned was large in amount because of the vastly increased amount of specific tuberculin protein and its prolonged reaction on highly sensitized membrane, induced by the wide-spread disease within the lung and pleura. It would seem probable that bacteria themselves must remain in the pleura or be added constantly to the

pleural fluids to produce an effusion. From material taken for biopsy, it is known that such a circumstance is possible, because Montgomery and one of us (W. S. L.) (7, 9) have repeatedly observed subpleural tubercles that were ulcerating through the pleura itself and depositing bacteria in the pleural space.

OBSERVATIONS

1. Intrapleural injection of nonspecific, inorganic, particulate material results in a pleural effusion containing the cellular constituents of the blood in either normal animals or animals sensitized by previous subcutaneous injection of living tubercle bacilli.

2. Intrapleural inoculation of tubercle bacilli does not cause an immediate inflammatory reaction in normal animals and consequently there is no pleural effusion.

3. Intrapleural inoculation of tubercle bacilli produces an acute inflammatory reaction in animals sensitized to tuberculin which is similar to that produced by inorganic particulate material. The components of the effusion, however, may vary with the numbers and virulence of the organism.

4. Old Tuberculin (specific protein) injected into the skin of sensitized animals produces an inflammatory reaction resulting in the exudation of serum and of cellular components of the blood. In normal animals there is no such inflammatory result.

5. Tuberculin introduced into the conjunctival sac of sensitized animals produces an inflammatory reaction in all respects similar to that produced by the subcutaneous injection of tuberculin into sensitized animals.

6. Intrapleural injections of nonspecific protein (skimmed milk) into either normal or sensitized animals do not produce any inflammatory changes or any effusion.

7. Intrapleural injection of specific tuberculoprotein (Old Tuberculin) into normal animals, as expected, produces no inflammatory changes and no effusion. Contrary to expectation, intrapleural injection of specific tuberculoprotein (Old Tuberculin) likewise produces no inflammatory changes and no effusion.

8. Tubercle bacilli introduced into the pleural sac of sensitized animals probably exert a foreign body reaction on the pleura, in many respects similar to that of inorganic particles of approximately the same size. An effusion regularly is produced.

CONCLUSIONS

Our experiments tend to prove that the rapid phagocytosis by polymorphonuclear and mononuclear phagocytic cells tends to localize bacilli and encourages the production of tubercles, and that later interference with the lymph current serves to immobilize the bacilli in the pleural space. With arrest of bacilli, lesions develop in both the parietal and the visceral pleura, the lungs are soon invaded and generalized tuberculosis develops. In the course of experimental tuberculosis, we had evidence that convinced us that tubercle bacilli are shed

into the sensitized pleura from subpleural tubercles that have ulcerated through the visceral pleura. In such cases pleural fluid occurs. We believe that the failure to produce an effusion following intrapleural injection of specific tuberculoprotein (tuberculin) may be due to rapid absorption, to dilution and distribution of the inoculum spread over the large pleural surfaces and to the absence of particulate material, and we are of the opinion that a sensitized pleura and living bacilli in the pleural space are requisite factors in the production of experimental tuberculous pleural effusion.

CONCLUSIONES

Estos experimentos tienden a demostrar que la rápida fagocitosis producida por las células fagocitarias, polimorfo y mononucleares, localiza los bacilos y fomenta la producción de tubérculos y que esta obstrucción de la corriente linfática sirve para inmovilizar los bacilos en el espacio pleural. Al estacionarse los bacilos, se presentan lesiones, tanto en la pleura parietal como visceral, los pulmones son invadidos rápidamente, y se produce tuberculosis general (granulia). En la evolución de la tuberculosis experimental, los datos obtenidos por los autores los han convencido de que se vierten bacilos tuberculosos en la pleura sensibilizada de los tubérculos subpleurales, que al ulcerarse atraviesan la pleura visceral. En esos casos se forman derrames pleurales. En opinión de los autores, cuando no se produce derrame consecutivo a la inyección intrapleural de tuberculoproteína específica (tuberculina), esto puede deberse a rápida absorción, dilución, o distribución del inóculo sobre las grandes superficies pleurales y la falta de partículas de material, lo cual justifica la opinión de que para la producción de derrame pleural tuberculoso experimental, son indispensables una pleura sensibilizada y la presencia de bacilos vivos en el espacio pleural.

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NOCARDIA[1] ASTEROIDES[2, 3]

Its Pathogenicity and Allergic Properties

CHARLES H. DRAKE[4] AND ARTHUR T. HENRICI[5]

The aerobic, acid-fast *Actinomycetales* not forming conidia have been placed in the genus *Nocardia*. These organisms are common in the soil and may be readily isolated by means of appropriate techniques as shown by Gordon and Hagan (1936) and others. The genera *Nocardia* and *Mycobacterium* are closely related both culturally and morphologically. Both genera are composed of predominantly oxidative organisms many of which have the ability to utilize paraffin hydrocarbons as a source of energy. The genus *Nocardia* is characterized by a branched mycelium which readily fragments into rods that are similar to cells of mycobacteria. Many species of acid-fast *Nocardia* have been described, but in many cases there is considerable question as to whether they deserve specific rank. Umbreit (1939) has reduced these to the status of strains or varieties of *N. asteroides*, but the species described by Henrici and Gardner (1921) and Huntemuller (1921) clearly differ from the others in their ability to liquefy gelatin and to give the tyrosinase reaction.

The type species, *Nocardia asteroides*, was isolated by Eppinger (1881) from a fatal case of brain abscess in man and was described under the name of *Cladothrix asteroides*. Although infections with this organism are not common, a number of cases have been reported. Most of these had a fatal outcome, and in many cases the infection was not diagnosed until autopsy. The majority of the reported infections involved either the lungs or the central nervous system, and in the latter case there is usually evidence that the disease originated in the pulmonary cavity and later metastasized to the brain and meninges. The clinical diagnosis is often tuberculosis and pathologically the lesions resemble those of tuberculosis, except that they tend to be more purulent in nature. The microscopical picture varies from abscesses to tubercle-like lesions. Older lesions and more chronic cases show the greatest resemblance to tuberculosis.

In addition to the biochemical, morphological and pathological similarities between *Nocardia asteroides* and the tubercle bacillus there is also a close antigenic relationship. Cross agglutination and cross complement-fixation between

[1] The more common name is *Proactinomyces* but, in view of a paper by Waksman and Henrici, now in press in the Journal of Bacteriology, showing that the correct name is *Nocardia*, this name is used in this paper.

[2] Condensed from a thesis submitted by Charles H. Drake in partial fulfillment of the requirements for the degree of Doctor of Philosophy at the University of Minnesota, Minneapolis, Minnesota.

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[5] With deep regret we announce the death, on April 23, 1943, of Dr. Arthur T. Henrici, Professor of Bacteriology at the University of Minnesota, coauthor of this paper. This loss will be greatly felt by all workers in the field of microbiology.

these organisms have been reported by Fritsche (1908), Claypole (1913), Henrici and Nelson (1922) and others. The possibility is at once apparent that with such an antigenic relationship an infection with either organism might give rise to an allergic state that would react to either tuberculin or to an analogous extract from *N. asteroides*. The frequency of positive tuberculin tests in adults invalidates any conclusions from the few tuberculin tests reported on human cases of infection with *N. asteroides*.

The question concerning the existence of a cross reaction in tuberculosis and infection with *Nocardia asteroides* can be resolved into the following five possibilities: (*1*) No allergic state is induced by infection with *N. asteroides* comparable to that in tuberculosis and hence the question of a cross reaction does not arise. (*2*) A specific allergic state is induced by infection with either organism, but the specificity is sharp and cross reactions do not occur. (*3*) The allergic state induced by infection with either organism gives rise to reactions with allergens from both organisms. (*4*) Tuberculosis gives rise to a specific allergic state reacting only to allergens from the tubercle bacillus, but these allergens also give rise to reactions in infections with *N. asteroides*. (*5*) The converse of the above: infection with *N. asteroides* gives rise to a specific allergic state reacting only to allergens from this organism, but these substances also give reactions in infections with the tubercle bacillus.

The present investigation was undertaken to see which of the above possibilities occur. Most of the investigations so far reported have made use of crude extracts, and it was hoped that by the use of purified fractions of the allergens more satisfactory results could be obtained.

It can be readily appreciated that an accurate and reliable skin test would greatly facilitate the diagnosis of actinomycosis caused by *Nocardia asteroides*. It is at once evident that a potentially pathogenic organism, common in the soil and morphologically and pathologically similar to the tubercle bacillus, might well be the cause of more infections than are reported.

MATERIALS AND METHODS

Organisms: The bacteria used in this investigation were the H37 strain of *Mycobacterium tuberculosis* and four different strains of *Nocardia asteroides*. Of the latter, one strain was isolated and identified by Kessel and was used for most of the investigation. Another strain was isolated and identified by Emmons, and the remaining two strains were isolated in this laboratory, one from autopsy material from a fatal human infection, and the other as an incidental finding on a plate streaked from a normal throat. The latter was most probably an air contaminant. All of these strains of *N. asteroides* were identical culturally and morphologically, and all were able to utilize paraffin as a sole source of carbon. The general properties of these organisms were in agreement with the published descriptions of *N. asteroides*, and all were acid-fast when an aqueous solution of sulfuric acid was used for decolorization. It should be emphasized that the acid-fastness of these bacteria is less than that of the tubercle bacillus and that the use of acid-alcohol will usually remove all of the

color from the cells, especially in slides made from cultures. In sputum the organisms are more resistant and will usually retain the color when treated with acid-alcohol.

Experimental animals: Adult rabbits and guinea pigs were used and were kept under the ordinary laboratory conditions.

Media: Cultures were maintained on standard nutrient broth and agar on which these organisms grow well. The tubercle bacilli were cultivated on glycerol-veal-infusion agar The ability of the actinomycetes to utilize paraffin was tested by growing the organisms in a mineral salts medium containing a glass rod dipped in paraffin. Two different liquid media were used in attempts to produce a product analogous to tuberculin. The first medium was composed of 1 per cent ammonium nitrate and 4 per cent glycerol in tap water and was later replaced by a 1 per cent peptone, 4 per cent glucose medium made up with tap water.

Injections: The cultures were suspended in sterile broth or mineral oil before injection. The coarser particles were removed by filtering through sterile gauze and, when necessary, dilutions were made in sterile broth. The animals were injected by various routes as explained later.

SKIN TEST SUBSTANCES

1. The tuberculin used was standard OT obtained from a commercial source and shown to give reactions in tuberculous animals. A constant dose of 0.1 cc. of a 1:100 dilution was employed.

2. A substance hereafter referred to as "asteroidin" was prepared from cultures of *Nocardia asteroides* grown on the liquid media described above. The method of preparation was that used for Old Tuberculin. Autoclaved cultures were evaporated to one-tenth volume on a water bath and filtered through Seitz or Berkefeld filters. It was found necessary to adjust the reaction of this material since the pH was as low as 3.5 on the ammonium nitrate medium and as low as 4.5 on the peptone medium. Skin tests with 0.1 cc. of this substance gave positive reactions in normal animals before neutralization but were well tolerated with minimal findings after the reaction was adjusted.

3. A polysaccharide fraction was prepared from the organisms by harvesting pellicles from liquid media and heating them to boiling in 1 per cent hydrochloric acid. As soon as the boiling point was reached the material was cooled, neutralized with dilute sodium hydroxide and filtered. Seven volumes of 95 per cent ethyl alcohol were added and the mixture was allowed to stand over night. The solution became milky after the addition of the alcohol and a flocculent precipitate slowly settled out. A few crystals of potassium acetate were usually added to speed up the flocculation. The tan colored precipitate was redissolved in a small quantity of distilled water and a few crystals of trichloracetic acid were added to precipitate any traces of protein that might be present. The solution was again filtered and another seven volumes of alcohol were added to precipitate the polysaccharide. The precipitate was again dissolved in water and precipitated with alcohol. The final product was a white or faintly tan

powder, soluble in water, which gave negative biuret and xanthoproteic tests for protein. Polysaccharide was also prepared by repeated alcoholic precipitation of the supernatant fluid from the protein fraction described below.

4. A protein fraction was prepared from *N. asteroides* by harvesting the pellicles from liquid media and draining off the excess fluid through filter paper. The bacteria were killed, dehydrated and defatted by repeated treatment with acetone. The treatment was continued until all of the reddish orange color was removed from the organisms and they felt dry and brittle to the touch. The residual acetone was evaporated and the organisms were ground to a fine powder in a refrigerated ball mill. The dry powder was extracted over night in the icebox with distilled water and the powder removed by filtration. Some of this extract was used as a skin test substance and is called crude extract. The protein was precipitated from the remaining liquid by the addition, drop by drop, of a concentrated solution of trichloracetic acid until no more precipitate was formed. The protein was collected by centrifuging and removing the supernatant fluid and was redissolved in distilled water made faintly alkaline with a trace of sodium hydroxide to neutralize residual trichloracetic acid. The protein was again precipitated by adding dilute hydrochloric acid until the isoelectric point was reached and the protein started to precipitate. The material was thrown down in the centrifuge and the protein again dissolved in alkaline distilled water. The protein was reprecipitated twice at the isoelectric point and preserved in the icebox as a sterile solution.

An attempt was made to prepare a protein fraction by the method given by Seibert (1934) for the preparation of PPD from cultures of the tubercle bacillus. This was unsuccessful, since no protein that can be precipitated with trichloracetic acid appears in the culture medium, such as is present in liquid cultures of *Mycobacterium tuberculosis.*

5. A skin test material was prepared by filtering broth cultures of *Nocardia asteroides.* This would contain any soluble, labile substances that would be destroyed by any of the other treatments.

6. Lipoid: The acetone extract of the organisms was filtered, evaporated to dryness and redissolved in acetone. After a second filtration the acetone was again allowed to evaporate and the remaining lipoidal material was used for skin tests and is referred to later as "lipoid."

7. Suspensions of both live and heat-killed organisms containing approximately one loopful in 0.1 cc. were used to test for the Koch reaction.

ANIMAL PATHOGENICITY

Although our primary purpose in this investigation was to study the cross allergic reactions, we encountered considerable difficulties in attempts to produce chronic or subacute infections in experimental animals, so that a study of the pathogenicity of these organisms became desirable. The usual references to the pathogenicity of *Nocardia asteroides* for experimental animals conveys the impression that this organism is quite pathogenic for laboratory animals and especially for guinea pigs. It was therefore rather surprising to find very

irregular results from guinea pig injections with all of the strains of the organisms that we used.

A careful survey of the literature shows that a considerable variation in pathogenicity has been reported by various authors. Nakayama (1906) was able to inject large doses of *Nocardia asteroides* intraperitoneally in guinea pigs with the production of a mild transitory peritonitis which was never fatal. MacCallum (1902) reported rapid death of guinea pigs and rabbits after intraperitoneal, subcutaneous or intravenous injections with wide-spread miliary lesions, especially after intravenous inoculation. Mice showed only local lesions and died with no changes in other organs. Kulikowska (1930) reported death in guinea pigs five to seventeen days after intraperitoneal inoculation and found acute peritonitis and many abscesses over the peritoneal surfaces of the organs. The infection was usually confined to the peritoneal cavity and the parenchyma of the organs was involved only in subacute cases. Kulikowska also reviewed the work of other authors and reported Burns and Grubauer as finding negative results from animal injections, while Mandelstamm and Kalinin, Huntemuller, Ascione and Gerbasi found death with toxaemic symptoms and no lesions. Bretey (1933) noted no ill effects in guinea pigs after three intraperitoneal injections of 10 mg. of live organisms. Bishop and Fenstermacher (1933), using a strain isolated from a bovine infection, found little virulence for cattle but were able to kill guinea pigs by intraperitoneal injections, although most of the lesions were confined to the abdominal cavity. Subcutaneous injections caused death with large local lesions and metastases to the internal organs. Gordon and Hagan (1936) found only minimal lesions in guinea pigs after injection. Goldsworthy (1937) found only slight lesions in guinea pigs injected with a culture isolated some time previously from a human infection. Rabbits developed granulomatous lesions in the lungs after intravenous inoculation. Goyal (1937) found only slight lesions in guinea pigs and none of the animals died after injections of *N. asteroides*.

This survey reveals that the reported pathogenicity has varied from no results to acute death with wide-spread lesions following animal inoculations. Several authors noted toxicity rather than invasive powers. In most cases intraperitoneal inoculations produced lesions confined to the abdominal cavity. In order to obtain a more complete picture of the pathogenicity of *Nocardia asteroides* for experimental animals a series of rabbits and guinea pigs were inoculated by various routes with the results summarized below. Studies were made on all four of the strains mentioned above.

Intraperitoneal injection of guinea pigs with *Nocardia asteroides* resulted in an acute peritonitis which was fatal when the dose was sufficiently large. The lesions are similar to those ordinary peritonitis, except that there is a greater tendency to the formation of walled-off abscesses in the omentum and on the peritoneal surfaces of the abdominal organs. The pancreas frequently shows extensive abscesses. If the animals survive a week or more, the lesions appear to be healing and the inflammatory reaction receding. In these animals death seems to be due more to the toxic effect of some absorbed substances than to

an actual infection. This is indicated by a fatty degeneration of the liver cord cells and areas of focal necrosis, although these findings may be attributable to an endemic paratyphoid infection in the animal colony. The lungs are usually congested and the lower poles may show abscesses from extension through the diaphragm. Commonly the omentum and several abdominal organs are fused together by abscesses and adhesions. In these cases the toxaemic symptoms may be due to the distorted anatomical relationships. The diaphragm is often fused with the liver by abscesses. In most cases the lesions do not appear to be severe enough to have caused death. These results were obtained with all of the strains, except that the Emmons strain seemed to give a more acute picture in guinea pigs.

Subcutaneous injections produce local abscesses which usually discharge pus and heal spontaneously. Small abscesses may resorb without rupturing. Spread from the local lesion was never observed. Large doses were occasionally followed by death several weeks later, but the only internal lesions found were the focal necrosis and fatty degeneration of the liver previously referred to. Repeated injection into the same area merely caused a repetition of the previous picture.

Intramuscular injections give the same results as do the subcutaneous injections, except that the abscesses are more likely to resorb without rupturing. No extension from the local area was observed, but large doses occasionally caused death with the same toxaemia as seen in the subcutaneous inoculations. Intravenous inoculations were not made in guinea pigs.

This evidence leads to the conclusion that these strains of *Nocardia asteroides* have a low invasive power for guinea pigs, but may cause death from peritonitis if injected intraperitoneally in sufficient dosage. Most of the lesions are confined to the abdomen and involve the peritoneal surfaces of the organs. There is some evidence that death is due to some toxic action, since the lesions are usually regressing at the time of death. The fatty degeneration and focal necrosis of the liver point to some toxic substance which must be heat labile and closely bound to the bodies of the organisms, since culture filtrates and heat-killed organisms are devoid of toxicity. Subcutaneous and intramuscular inoculations cause only local lesions from which extension does not occur. Large doses may be followed by death of the animal with toxic lesions of the liver.

Rabbits were also tested in our attempts to produce a subacute or chronic infection which might lead to sensitization. It was realized that rabbits are not entirely satisfactory for the study of bacterial allergy by means of skin tests, since their response to tuberculin after infection with tubercle bacilli is much less marked than is the case with guinea pigs. Goyal (1937) flatly states that rabbits are not suited for the study of allergic skin tests. However, it was hoped that a more satisfactory disease state could be induced, since several authors have reported the formation of granulomatous lesions in the lungs following intravenous inoculation.

The intravenous inoculation of rabbits with *Nocardia asteroides* results in a generalized infection with miliary lesions in all parts of the body, including the

skeletal muscles. Death occurs within two to twenty-four days depending upon the size of the inoculum. Most of the animals died within a week after injection. Animals surviving longer than a week showed abscesses the size of a match-head. It was noted that infection by this route was an "all or none" phenomenon, in that the animal either died with acute lesions or survived indefinitely with no signs of the infection. Above the critical dose all of the animals died, but the injection of smaller quantities caused no reaction whatsoever and had no effect on the later injection of larger doses. An attempt was made to raise the virulence of the organisms by animal passage in the hope that a smaller quantity of a more virulent strain might result in the formation of a few progressive lesions. Although the virulence could be raised so that the injection of 0.2 cc. of a 1:100 dilution of a twelve-day broth culture would cause death in ten days with wide-spread miliary lesions, no success was had in attempts to produce a few progressive lesions. Out of more than 100 animals injected intravenously, only one rabbit showed any chronic lesions. This animal developed a testicular abscess which gradually enlarged with a local extension of the lesion over a period of several months until the testis was enlarged to approximately ten times its original size. The animal was found dead with a rupture of the testis and showed purulent granulomatous lesions with slight extension into the surrounding tissues and a few lesions along the spermatic cord extending into the lower abdomen.

Subcutaneous and intramuscular injections of rabbits gave the same results as in guinea pigs. The abscesses either ruptured and healed or resorbed. None of the rabbits died as a result of these local injections and no extension of the local lesion occurred. Palpation of the regional lymph nodes showed no apparent involvement.

Intraperitoneal inoculations seemed to have little effect on rabbits. If the animals were sacrificed at intervals, minimal abscesses could be found in the omentum and mesentary as well as a few small areas of peritonitis. After longer intervals only slight adhesions and occasional small, walled-off abscesses could be found.

It was concluded from these results that the strains of *Nocardia asteroides* studied had little invasive power for rabbits, but that the intravenous inoculation resulted in the formation of miliary abscesses throughout the body. Below a certain infective dose no lesions were found. All of the strains showed the same reactions, except that the Emmon's strain gave no lesions, even though it was the most pathogenic strain for guinea pigs. Death was probably due to the destruction of tissue by the immense numbers of miliary lesions and perhaps to the action of some toxic factor in the organisms. Repeated injections into the same area or a previous subinfective intravenous injection had no effect on the results of a later injection. Microscopically the lesions were abscesses with no formation of granulomatous tissue, except that a few giant cells were occasionally seen in the walls of the old abscesses. Gram-Weigert stains showed that the organisms were present in the lesions as branched mycelium and in the older lesions were found mostly at the periphery of the abscess. Occasional

small clumps of organisms were found but no granules such as occur in typical actinomycosis.

ALLERGIC PROPERTIES AND CROSS-SENSITIZATION

A few rabbits and guinea pigs injected by various routes with broth cultures of the organisms survived long enough so that skin tests could be tried, although no very satisfactory results were obtained. These experiments carried out at the beginning of the investigation did yield information concerning the toxic nature of the organisms. The intra- and subcutaneous injection of living or heat-killed organisms in attempts to produce Koch reactions gave identical results in control and infected animals, except that the infected animals occasionally showed a flushed zone surrounding the injected area. In the light of later findings this is interpreted as due to the polysaccharide present in the organisms. It should be pointed out that a definite inflammatory reaction occurred to both the living and heat-killed organisms in both infected and control animals. This reaction appears to be due chiefly to the lipoids present in the organism since the injection of lipoid gave rise to an inflammatory reaction almost identical with that of the whole organisms. This reaction was of equal severity in both infected and control animals. If any degree of sensitization had been produced the animals should have shown a Koch reaction, but no significantly greater reactions were obtained in the infected animals. The injection of protein and asteroidin gave no reactions. The injection of polysaccharide, crude extract and culture filtrate occasionally gave small flushed areas in the infected animals when the controls were negative. The toxicity of the lipoid and whole organisms has not previously been noted and may well explain some of the conflicting reports in the literature, especially in those cases where controls were not used. Tuberculin tests were consistently negative in these animals.

Since simple injections of the animals by any route tried did not result in any subacute or chronic infection, bacterial allergy could not be satisfactorily tested in such animals. However, we were successful in inducing chronic lesions by the intratesticular injection of oil suspensions of *Nocardia asteroides*. This method has been used for the study of allergy in tuberculous guinea pigs and has been found to produce rapidly a high degree of sensitivity in these animals. This is probably due to the production of granulomatous tissues as a reaction to the oil, which allows only a slow absorption of bacterial products and at the same time partially protects the organisms against the defense mechanisms of the body.

A series of rabbits and guinea pigs were injected intratesticularly with 0.1 cc. of an oil suspension of *Nocardia asteroides*, containing approximately one loopful of organisms removed from an agar slant. These animals were skin tested at intervals with the materials described previously. Skin tests were described as positive only when they gave a distinctly greater reaction than control animals tested with twice the dosage. Definite and consistent sensitization was demonstrated in animals inoculated with oil suspensions.

Five rabbits were injected intratesticularily with 0.1 cc. of an oil suspension of *Nocardia asteroides*, containing approximately one loopful of organisms. Skin tests were negative to tuberculin and asteroidin before the injection and again ten days after the animals were infected. Twenty-six days after the injection the tuberculin test was negative and the skin test with asteroidin gave a slight flushed area. Control animals were negative. Thirty days after infection skin tests with crude extract gave distinct, well developed reactions consisting of oedematous pink lumps one inch in diameter which receded after one week. Control animals showed no reactions and the tuberculin test was negative in both groups. Thirty-eight days after infection, skin tests with culture filtrate were negative. Seventy-three days after infection tuberculin gave negative skin tests as did crude extract from heat-killed organisms, but crude extract from unheated organisms gave distinctly positive reactions. These consisted of flushed areas appearing in twenty-four hours and increasing to a diameter of one inch in forty-eight hours. The centre of the flushed area was a three-fourths inch lump with a necrotic centre. Control animals gave no reactions to any of the three test substances. Unfortunately all of these animals were lost through a laboratory fumigation before further tests could be carried out with purified protein and polysaccharide.

The results of the above tests are very suggestive and allow several conclusions to be drawn. Tuberculin tests were consistently negative. Skin tests with culture filtrate were negative and tests with asteroidin gave only slight flushed areas. Skin tests with crude extract from heated organisms gave no reactions, but tests with crude extract of unheated organisms gave definite, positive reactions consisting of a good-sized flushed area with a central lump and necrosis. The reaction did not reach its height until forty-eight hours after the injection.

Ten guinea pigs previously shown to give negative reactions to the skin test materials were injected intratesticularly with 0.1 cc. of an oil suspension of *Nocardia asteroides* containing one loopful of organisms. Twenty-five days after infection the tuberculin test was negative, but there was a very slight reaction to asteroidin. Thirty days after infection the tuberculin test was negative, but the injection of 0.1 cc. crude extract gave a raised flushed area, half an inch in diameter, that receded after four days. Control animals gave no reactions. Thirty-eight days after infection the animals gave no reaction to culture filtrate and after forty-eight days gave only a slight reaction to asteroidin. Fifty-four days after infection the tuberculin test was negative but 0.1 cc. culture filtrate gave a three-fourths inch, slightly raised, flushed area that disappeared in one week. Seventy-three days after infection the tuberculin test was still negative as were the skin tests with crude extract from heated organisms, but the injection of 0.1 cc. of crude extract from unheated organisms gave a three-fourths inch, raised, red area with a necrotic centre. The height of the reaction was not reached until the second day after the injection. Control animals gave no reactions. Eighty-seven days after infection the animals were skin tested with 0.1 cc. asteroidin, 0.25 mg. protein and 1 mg. polysaccharide.

The asteroidin gave only a slightly flushed area. The polysaccharide gave a two-thirds inch, slightly raised, flushed area that increased only slightly on the second day and faded away after five days. The protein gave a dime-sized, raised red area that increased in forty-eight hours to almost one inch in diameter with a yellowish necrotic centre and persisted for one week. Control animals showed no reactions. Ninety-four days after infection the tests with protein, polysaccharide and tuberculin were repeated. The tuberculin tests were negative, but the reactions to the protein and polysaccharide were the same as on the last tests. Control animals gave no reactions. One hundred and five days after infection, the animals were skin tested with 0.1 cc. each of crude extract, culture filtrate and asteroidin. The culture filtrate and asteroidin gave slight, flushed areas, but the crude extract gave a three-fourths inch, red lump that increased in forty-eight hours to a one-inch lump with slight central necrosis. The reaction healed in eight days and the control animals remained negative. One hundred and twenty days after infection, the skin tests with protein, polysaccharide and tuberculin were repeated. The tuberculin test was negative, but 0.25 mg. protein gave a dime-sized, raised red area that increased on the second day to almost one inch in diameter and developed a necrotic centre. The reaction persisted for nine days. One milligram of polysaccharide gave a three-fourths inch, red flush that increased only slightly on the second day and disappeared after five days. Control animals gave no reactions. The animals were sacrificed one hundred and twenty-eight days after infection and, at autopsy, the testes were enlarged and contained granulomatous tissue with many necrotic areas. There were a few small abscesses along the spermatic cord but no other lesions were found. The results of these tests in guinea pigs confirm and extend the observations made in rabbits.

One rabbit, mentioned earlier, developed a testicular abscess following intravenous inoculation and survived for a considerable period with a slowly developing lesion. Fifty-one days after infection this animal gave a negative test to asteroidin. One hundred and fifteen days after infection a skin test with 1 mg. polysaccharide gave a three-fourths inch flushed area that faded after three days. The animal was again tested with polysaccharide one hundred and thirty-three days after infection with the same result. Two hundred and twelve days after infection the animal was skin tested with 0.25 mg. protein and 0.1 cc. tuberculin. The tuberculin test was negative, but the protein gave a dime-sized, pale red raised area with a dark centre. On the second day the centre became necrotic and the reaction persisted for one week.

The evidence from these experiments points to several conclusions. Rabbits and guinea pigs, sensitized by the intratesticular injection of oil suspensions of *Nocardia asteroides*, develop a bacterial allergy to the organism. Skin tests with asteroidin and culture filtrate give either no reaction or only slight reactions consisting of small flushed areas. Skin tests with lipoid and live or heat-killed organisms give rise to acute inflammatory reactions in both control and infected animals. Crude extract from heat-killed organisms gives no reaction, but skin tests with crude extract from unheated organisms give definite reactions in in-

fected animals but no reactions in controls. The reactions consist of a raised, red area which enlarges on the second day, persists for about one week and often shows central necrosis. The polysaccharide fraction obtained from crude extract gives slightly raised, fair-sized flushed areas that reach their height in twenty-four hours and disappear in a few days. Necrosis or considerable swelling of the area is never observed and no reactions are produced in controls. The protein fraction of crude extract gives raised, swollen areas that increase in size on the second day, persist for a week, and usually show central necrosis. This material has no effect on normal animals. The reactions to protein are sharply demarcated from the surrounding skin, while the reactions to the polysaccharide and crude extract have no sharp border and fade into the normal skin.

Five guinea pigs were injected intratesticularly with 0.1 cc. of an oil suspension of *Mycobacterium tuberculosis*, H37 strain, containing one loopful of organisms. One month later the animals had developed sufficient allergy so that the injection of 0.1 cc. of a 1:100 dilution of tuberculin gave a strongly positive reaction with a central necrotic area one-third inch in diameter. These animals were then tested with the skin test materials described previously, using the same dose and twice the dosage used on the animals infected with *Nocardia asteroides*. Neither protein, polysaccharide, crude extract, asteroidin nor culture filtrate gave any reaction, showing a complete lack of sensitivity of the tuberculous animals to the allergens from the actinomycete. The Koch reaction carried out with live suspensions of *Nocardia asteroides* gave no greater reactions than those in the controls.

DISCUSSION

The general problem of the antigenic relationship between the mycobacteria and the acid-fast actinomycetes has been studied by a number of authors. In addition to the work of Fritsche, Claypole, and Henrici and Nelson mentioned earlier, Bretey (1933) found some complement fixation with tuberculosis serum and antigens from *Nocardia asteroides*, although less than with the homologous antigen. The same author, using precipitin tests, found reactions to antigens from the tubercle bacillus, other acid-fast bacilli and *N. asteroides* to occur at the same dilutions, using antiserum prepared against the tubercle bacillus. However, antiserum against *N. asteroides* reacted only with the homologous antigens. Goyal also noted an antigen common to both acid-fast bacilli and *N. asteroides* by means of complement fixation, agglutination and precipitin tests. Several authors noted that these organisms were poor antigens *in vivo*.

The direct question of cross allergic reactions has also been studied by several workers. Nakayama noted an interesting allergic phenomenon in that guinea pigs receiving a second intraperitoneal injection of *Nocardia asteroides* one week after the primary injection died within twenty-four hours. Both injections had to be made intraperitoneally or death did not occur. If tubercle bacilli were used for the second injection, acute death did not occur, but if tubercle bacilli were used for the primary injection and *N. asteroides* for the second injection, the animals also died within twenty-four hours. Bretey (1933) used several

ways of studying allergic cross reactions in guinea pigs injected with tubercle bacilli and acid-fast actinomycetes. Tuberculin and analogous extract were prepared and tested on animals injected with the two organisms. Tuberculin gave no reactions in animals injected with the acid-fast actinomycetes, but the extract from the actinomycetes gave reactions in animals injected with these organisms and to a lesser extent in tuberculous animals. The extract of the actinomycetes was able to cause death in some tuberculous animals if injected in sufficient dosage. Animals injected with avian tubercle bacilli did not give a Koch reaction to cells of *N. asteroides* but animals injected with bovine tubercle bacilli did. Animals infected with *N. asteroides* gave no reaction to the cells of bovine tubercle bacilli but did to the homologous organism. Bishop and Fenstermacher (1933) found negative tuberculin tests in calves and rabbits infected with *N. asteroides*, but one of two guinea pigs did give a positive tuberculin test. Neither animal showed lesions on autopsy. Mathieson, Harrison, Hammond and Henrici (1934) found no positive tuberculin tests in rabbits or guinea pigs injected with *N. asteroides* or *N. gypsoides*. Vaccination with these organisms had no effect on subsequent infections with tubercle bacilli. They quote Zupnik as finding no reaction to the injection of large quantities of tuberculin in animals immunized against acid-fast actinomycetes. Goyal concluded that guinea pigs, sensitized with actinomycetes, reacted to the intracutaneous injection of tuberculin to almost the same extent as to the homologous extract. This sensitivity developed slowly and was sometimes shown by normal animals that had been kept in the laboratory. Tuberculous animals reacted feebly to the injection of extracts of the actinomycetes. Sensitized animals gave an irregular temperature rise to the injection of tuberculin or extracts of the actinomycetes. The intracorneal injection of extracts of acid-fast actinomycetes in tuberculous rabbits rarely gave any reactions. The Koch reaction was negative using *N. asteroides* in tuberculous animals, but the injection of either heat-killed tubercle bacilli or *N. asteroides* in animals sensitized to the latter gave almost identical reactions. The intraperitoneal injection of living actinomycetes had no effect on tuberculous guinea pigs.

Several criticisms can be leveled at some of these reports. In a few cases the use of controls was not mentioned. In other cases the control animals occasionally gave positive reactions, and all of the animals were kept in the same quarters with tuberculous animals. In many of the reports the differences between the control and infected animals were quantitative rather than qualitative, which is open to criticism unless the differences are very marked. Our findings have indicated that living or heat-killed organisms or the lipoids extracted from the organisms cause considerable inflammatory reactions following the local injection of these materials in normal animals. Preparations analogous to tuberculin are very acid in reaction and also give rise to reactions in normal animals unless neutralized before injection. These toxic and irritant actions may well explain the conflicting reports in the literature.

In our attempts to repeat the work of the above authors we found that it was too difficult to interpret the results of the skin tests if whole cells were used.

Culture filtrates have little activity probably because of the lack of protein as is found in culture filtrates of the tubercle bacillus. Crude extract of heated organisms has little activity probably because the allergens are thermolabile. However crude extract of unheated organisms gives rise to skin test reactions in animals sensitized by the intratesticular injection of oil suspensions of *Nocardia asteroides*. This extract can be fractionated into a polysaccharide and a protein fraction. The polysaccharide gives skin tests consisting of reddened areas that develop within twenty-four hours, subside after four days and never show necrosis. The edges of the reaction are not sharp and fade into the surrounding normal skin. The protein gives skin tests that consist of smaller swollen areas which develop more slowly, not reaching their height until forty-eight hours after the injection. The centre usually shows a necrotic area and the whole reaction is more sharply marked off from the normal skin. Animals sensitized to *N. asteroides* are not sensitive to tuberculin and give no reactions to this substance. Tuberculous animals are not sensitive to the injection of the substances that do give reactions in the animals infected with the actinomycetes.

We have been able to demonstrate a specific allergy against *Nocardia asteroides* that is very similar in its general characteristics to tuberculous allergy. However, this allergic state is more difficult to induce in experimental animals than is tuberculous allergy. Two reasons might be suggested for this. The organisms show less true pathogenicity for animals and are almost devoid of invasive power so that chronic lesions are not as readily produced. There is abundant evidence in the literature to suggest that bacterial allergy is connected with chronic lesions and granulomatous tissue structure. In culture media inoculated with tubercle bacilli, specific protein appears in the medium, but this is not true with cultures of *N. asteroides*. If this is also true in the body one could easily understand why allergy was less readily induced against the acid-fast actinomycetes. The allergens of *N. asteroides* differ from those of the tubercle bacillus in that they are thermolabile since both asteroidin and extracts of heat killed organisms have little or no activity.

CONCLUSIONS

1. *Nocardia asteroides* has little invasive power for rabbits and guinea pigs. Large doses intraperitoneally in guinea pigs, smaller doses intravenously in rabbits produce death with acute peritonitis and multiple miliary abscesses, respectively. But smaller doses produce neither lesions nor death. Subcutaneous and intramuscular injections did not spread, but healed. All attempts to produce a progressive disease similar to tuberculosis failed.

2. An allergic state in rabbits and guinea pigs against *N. asteroides* could be induced with regularity only by the intratesticular injection of oil suspensions of live organisms.

3. The allergic state could be demonstrated by skin tests with extracts of powdered organisms or by purified protein and polysaccharide.

4. The reaction to the crude extract and to protein consists of a slowly developing swollen area with central necrosis that resembles the tuberculin test in tuberculous animals; whereas the reaction to the polysaccharide consists of a

nonelevated reddened area that reaches its height in twenty-four hours and disappears after four days. Central necrosis never occurs.

5. The allergens are thermolabile and closely bound to the bodies of the organisms since asteroidin, culture filtrates and extracts of heated organisms are without effect.

6. The cells of *N. asteroides* are not suitable for use as a skin test substance since they are toxic and give rise to reactions in normal animals. Similar reactions occur with lipoids extracted from the organism.

7. The allergy induced against *N. asteroides* is specific and there are no cross reactions to tuberculin. Tuberculous animals with a high degree of allergy as shown by the tuberculin test give no reaction to the allergens of *N. asteroides*.

CONCLUSIONES

1. El *Nocardia asteroides* muestra poca facultad invasora para los conejos y los cobayos. Las dosis masivas empleadas intraperitonealmente en los cobayos, y las más pequeñas intravenosamente en los conejos producen la muerte asociada a peritonitis aguda y múltiples abscesos miliares respectivamente, pero las muy pequeñas no producen ni lesiones ni mortalidad. Las inyecciones subcutáneas e intramusculares no se propagaron sino que cicatrizaron. Todos los esfuerzos realizados para producir una enfermedad evolutiva semejante a la tuberculosis fracasaron.

2. Sólo pudo provocarse con regularidad un estado alérgico en los conejos y cobayos contra el *N. asteroides* por medio de la inyección intratesticular de suspensiones oleosas de microbios vivos.

3. Pudo demostrarse la existencia del estado alérgico mediante cutirreacciones ejecutadas con extractos de microbios pulverizados o con proteína purificada o un polisacárido.

4. La reacción al extracto bruto y a la proteína consiste en una zona edematizada que se desarrolla lentamente con esfacelo central y que se parece a la producida por la reacción a la tuberculina en los animales tuberculosos, en tanto que la reacción al polisacárido consiste en una zona enrojecida y sin elevación que alcanza habitualmente su acmé en 24 horas y desaparece al cabo de cuatro días sin mostrar jamás esfacelo central.

5. Los alergenos son termolábiles y están íntimamente unidos al cuerpo de los microbios, dado que no surten efecto la asteroidina, los filtrados de cultivos y los extractos de gérmenes calentados.

6. Las células del *N. asteroides* no se prestan para empleo como sustancia de cutirreacción, pues son tóxicas y provocan reacciones en los animales normales, observándose reacciones semejantes con los lipoides extraídos del microbio.

7. La alergia evocada contra el *N. asteroides* es específica y no va acompañada de reacciones cruzadas a la tuberculina. Los animales tuberculosos dotados de alergia elevada según revela la reacción a la tuberculina no reaccionan a los alergenos del *N. asteroides*.

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AMERICAN TRUDEAU SOCIETY

Report of the Committee on Tuberculosis Sanatorium Standards

Dr. Victor F. Cullen, *Chairman*
Dr. John F. Busch — Dr. Ralph Horton
Dr. Fred H. Heise — Dr. John Nicklas
Dr. John W. Towey

I hand you a report of the Committee on Tuberculosis Sanatorium Standards of the American Trudeau Society covering the activities of the past year.

I met Doctor Guild at Memphis where we discussed a program and decided to send out to representative sanatoria a questionnaire, requesting information on the short cuts and expedients they have developed to take care of the emergency that exists.

The full Committee met in New York City on February 8, 1943, at the Hotel Pennsylvania. Attending this meeting we also had representatives of the Office of Civilian Defense, the United States Public Health Service and the American Red Cross. Doctor Guild, Executive Secretary of the American Trudeau Society, and Dr. Charles E. Lyght, Director of Health Education, National Tuberculosis Association, attended. Dr. J. Burns Amberson, Jr., President, National Tuberculosis Association, was present for the afternoon session.

The Committee went on record as approving the record forms prepared by the Potts Memorial Institute for use in sanatoria and clinics. It was brought out that if the recommendation of the Society was desired, the request must be presented directly to the Council.

Mr. Richard Mackenzie, hospital administrator and management consultant, was also present and gave a brief résumé of his previous experience in the field of sanatorium work. He said that his aims in surveying sanatoria were to save money, to develop a more uniform administrative procedure and to investigate institutions in order to correct defects and increase their efficiency. This consultant is now making a study of a large county institution in New York on a demonstration basis, and his findings will be made available to the Committee on Tuberculosis Sanatorium Standards.

Doctor Dearing, representing the Office of Civilian Defense, gave a brief statement of the plans under consideration for civilian care in the event of an air raid or some similar catastrophe. Doctor Clark, Chief of the Hospital Division of the Office of Civilian Defense, explained that arrangements have already been made with some of the hospitals and sanatoria in the coastal regions. The Office of Civilian Defense does not advise the removal of patients from the sanatoria because many in England felt that this was the cause of the increase in tuberculosis there, and we do not want to subject the civilian population in this country to any such hazard. Doctor Clark said that tuberculosis sanatoria could be used as emergency base hospitals by doubling up and doing a little

crowding, and his office recommends a ratio of one emergency bed for every four general hospital beds. Beds, mattresses and other supplies are being held in O.C.D. warehouses ready for rapid delivery to institutions when needed. This emergency organization will also supply a limited number of physicians and nurses to be called when needed. Of the 179 institutions asked to coöperate in this plan, 150 had already responded. The institutions will be paid at the rate of $3.75 per day for all patients put into the emergency beds so that the sanatoria making beds available will not bear the financial burden of extra equipment.

The Committee believed this information should be disseminated to sanatorium superintendents, and Doctor Dearing was asked to prepare a news release and forward it to the National office which would distribute the information along with a covering letter to sanatorium administrators throughout the country. It was further recommended that the material be published in the AMERICAN REVIEW OF TUBERCULOSIS. A vote of thanks was extended by the Committee to Doctor Dearing and the Office of Civilian Defense.

The Committee now has two problems: (*1*) a long-range one for developing minimum standards for sanatoria; (*2*) a short-range, or war-time problem, as to what should be done to alleviate the results of shortages in personnel and equipment.

These problems were freely discussed and it was the consensus of the Committee that a subcommittee on sanatorium planning and construction should be appointed and that the chairman of this subcommittee work with the Committee on Tuberculosis Sanatorium Standards and attend its meetings. It was also recommended that a nurse be appointed by the National Tuberculosis Association to the subcommittee to act as a consultant.

Doctor Sharp of the United States Public Health Service brought before the Committee the fact that we are witnessing in Europe a rather sharp increase in the incidence of tuberculosis and that in all probability we shall have the same thing in this country. The United States Public Health Service, working through local health departments, is X-raying industrial workers on a voluntary basis, and Doctor Sharp recommended that the American Trudeau Society Committee on Tuberculosis Sanatorium Standards stress the advisability of X-raying industrial employees and also determine the disposition of new cases discovered when no sanatorium facilities are available.

A vote of thanks was extended to Doctor Sharp and the United States Public Health Service. It was recommmended to the Executive Committee that the importance of X-raying industrial workers be publicized through the usual channels and that a release from the United States Public Health Service be included in the AMERICAN REVIEW OF TUBERCULOSIS.

The report of the returns of the questionnaires sent out to the various sanatoria was compiled by the Statistical Service of the National Office by Miss Dempsey. It was recommended that the findings be published in the American Trudeau Society section of the AMERICAN REVIEW OF TUBERCULOSIS and that a copy

of the final report be sent to the Surgeon General of the United States Public Health Service and to the Chief Medical Officer of the Office of Civilian Defense.

It was also recommended by the Committee that a resolution be prepared emphasizing the necessity of adequate sanatoria budgets, maintenance of sufficiently high standards to safeguard against the retardation of the tuberculosis campaign, accelerated case-finding programs and good home-nursing service.

The Committee discussed the prohibition against the use of Red Cross nurses' aides in tuberculosis institutions and, on motion, the Committee voted that, inasmuch as Red Cross aides were used in general hospitals and exposed to communicable diseases in institutions where they are not sure that patients are free from tuberculous infection, the Committee feels it is unfair to prohibit the use of nurses' aides in tuberculosis sanatoria. It was further suggested that a chest examination should be required and an age limit set for Red Cross nurses' aides before they can serve in tuberculosis hospitals.

The Committee extended a vote of thanks to Miss Dempsey and Doctor Guild for their work in the preparation of the report.

The Committee feels that something has been accomplished, but that it is not the time to present a final report. Therefore, we recommend that the Committee be continued and that one meeting be held during the next fiscal year.

AMERICAN TRUDEAU SOCIETY

Report of the Membership Committee

Dr. Hugh B. Campbell, *Chairman*
Dr. Byron F. Francis Dr. Paul P. McCain

I regret that the distances which separate us have prevented the members of the Membership Committee of the American Trudeau Society from holding any meetings during the year. Again it has been necessary to discuss matters through correspondence. Dr. Paul McCain has been active in the southeastern district of the United States. I have no doubt that Dr. Byron F. Francis of Seattle has stimulated the interest of medical practitioners in the northwest.

The selection of the membership certificate, in which the Membership Committee participated, has evidently met with general approval. Its simplicity and artistic arrangement have also excited favorable comment from many not connected with the Society.

The *Membership Roster*, made available the past year, fills a long needed want. Not only does it register the names of those physicians affiliated with the Society, but it is a source of comfort and satisfaction to any practitioner desiring to refer his patients to competent advisers and pneumothorax experts in other parts of the country.

The membership continues to grow despite the disrupting factors incidental to the war effort. Recent figures from the central office demonstrate that growth:

Total enrollment March 31, 1943	1,647
Total enrollment March 31, 1942	1,237
Increase	410
Applications for emeritus membership	2
Military Service	
Members paying no dues	174
Members paying $7.00 for REVIEW	40
Cancelled and resigned (1941 and 1942)	23
Deceased (1941 and 1942)	22

I am sure the other members of the Committee join with me in giving the major part of the credit for the Society's growth to the zealous efforts of the New York office group. Through the initiative and drive which they have continually manifested, opportunities to associate themselves in membership have been given to those graduates in medicine, either by group or individually, interested in any phase of the tuberculosis problem. Nor have these efforts been confined to the United States alone. Examination of the *Roster* reveals a wide-spread membership throughout the Western Hemisphere and in the Insular possessions in the Pacific.

AMERICAN TRUDEAU SOCIETY

Report of the Committee on Postgraduate Medical Education

Dr. James J. Waring, *Chairman*

Dr. Grover C. Bellinger	Dr. Edward Kupka
Dr. Chesley Bush	Dr. Rufus R. Little
Dr. Henry D. Chadwick	Dr. C. Howard Marcy
Dr. Loren L. Collins	Dr. Howell S. Randolph
Dr. Leroy U. Gardner	Dr. Hyman I. Spector

Dr. James C. Walsh

The newly constituted Committee on Postgraduate Medical Education of the American Trudeau Society has not found it possible to have a meeting to this date. All business of the Committee has, therefore, been conducted by correspondence. The Chairman has had interesting and helpful letters from all members of the Committee.

The situation as to postgraduate education in tuberculosis would appear to be influenced by the following considerations:

1: The needs of the Army and Navy have drawn off almost completely the men who might have been interested in taking a postgraduate course of training in tuberculosis.

2: The men who remain are so overwhelmed with the responsibilities of civilian practice that they cannot take residential work in any institution anywhere, except under unusual circumstances.

3: On the other hand, many institutions have already indicated that they are in great need of temporary or permanent residential assistance.

4: It does not appear that the Army and Navy are making any special effort for formal training in tuberculosis of the men inducted into either arm of the service.

5: It would seem that this Committee can, during the duration of the war, spend its time and effort most usefully in the following directions:

(*a*) Encourage the setting up of formal training in tuberculosis in both the Army and Navy.

(*b*) Canvass the institutional field very carefully with a view to accrediting certain institutions for a short-term residence in tuberculosis.

(*c*) When hostilities cease, a large number of doctors will be discharged from the Army and Navy. At that moment the National Tuberculosis Association and the American Trudeau Society should be prepared, by adequate publicity, to bring to the attention of these men the importance of and the timeliness of taking a short-term course in tuberculosis before resuming private practice.